JOURNAL OF AGRICULTURAL RESEARCH

VOL. 74 WASHINGTON, D. C., MAY 1 & 15, 1947 NOS. 9 & 10

INHERITANCE OF REACTION TO RUST IN FLAX[1]

By H. H. FLOR

Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

All varieties of cultivated flax tested have been found to be susceptible to one or more of the races of flax rust (*Melampsora lini* (Pers.) Lév.) occurring naturally in North America or South America (*3*, *10*).[2] Although no variety that is resistant to all races is known, it may be possible to develop such a variety by combining through hybridization various resistant or immune factors possessed by certain varieties that serve to differentiate the physiologic races. Races capable of attacking all varieties are not known in any one flax-producing region; therefore it is not yet necessary to incorporate resistance to all known races into a variety developed for a particular region. Nevertheless, a program to develop rust-resistant varieties of flax would be facilitated by knowledge of (1) the pathogenic capacities of the physiologic races of *M. lini* occurring or likely to occur in the region with which the investigator is concerned and (2) the interaction between the factors conditioning resistance to or immunity from rust. The present paper is concerned primarily with the latter phase of such a program.

REVIEW OF LITERATURE

Henry (*7*) found that immunity from a North American rust collection was dominant and conditioned by single pairs of factors in Ottawa 770B and Bombay and by 2 pairs in an Argentine selection. In a study on the inheritance of reaction to North American races of rust in crosses involving 17 varieties of flax, Myers (*8*) accounted for his results by assuming factors in 2 allelic series. Immunity was dominant and conditioned by the duplicate factors *L* and *M*. The less resistant infection types were hypostatic to the more resistant ones, the factors l^n and m^n conditioning near immunity and l^r and m^r resistance. Susceptibility was conditioned by the recessive factors *ll* and *mm*. In a study on the inheritance of rust reaction in a cross between Buda and J. W. S., Flor (*4*) found that each of these varieties possessed a pair of factors which conditioned immunity from certain

[1] Received for publication Jan. 17, 1946. Cooperative investigations of the Division of Cereal Crops and Diseases and the North Dakota Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 262.

races of rust and were allelomorphic to the factors for immunity in Ottawa 770B. He also found that a single pair of factors conditioned resistance to one race, semiresistance to a second, moderate susceptibility to a third, and high susceptibility to a fourth.

In studies on the inheritance of factors conditioning resistance to disease in plants, it usually has been assumed that the two parent varieties have identical factors for resistance when their hybrid progeny yield no segregates susceptible to a race of the pathogen to which both parents are resistant (*8*, *9*). However, this may not necessarily be true if the factors are allelomorphic. Myers (*8*) considered the immune factors possessed by Ottawa 770B and C. I.[3] 438 (selection Minn. 25–107 from Minn. 281) to be identical. Flor (*3*) subsequently found that C. I. 438 was highly susceptible to races 19 and 20 of *Melampsora lini*, whereas Ottawa 770B was immune from these races. Ottawa 770B and C. I. 438 apparently possess duplicate allelomorphic factors for immunity from certain North American races.

MATERIALS AND METHODS

It was originally planned to study the inheritance of rust reaction in flax (*Linum usitatissimum L.*) by crossing in all possible combinations the 11 varieties used to differentiate physiologic races of rust (*3*). During the later phases of the study hybrid rust cultures (*6*) became available; by use of these, 6 varieties from the group that had been classed as immune from or resistant to North American races and susceptible to South American races (*3*) were found to possess rust-conditioning factors distinct from each other and from those of the 11 rust differentials. The varieties Newland, Bolley Golden, Italia Roma, Leona, and Tammes Pale Blue had sharp differences between resistant or immune and susceptible reactions and have been included in this study. The reaction of the sixth variety, Light Mauve (C. I. 379–1), although distinct from each of the others, was one of degree and subject to considerable variation by changes in environment. Because of this variability, a genetic study of the factors for rust reaction possessed by this variety was not undertaken. In addition, limited studies were made on the inheritance of rust-conditioning factors in C. I. 416–3 (Pale Verbena) and C. I. 438, varieties studied by Myers (*8*), in Rio, a variety developed at the North Dakota Agricultural Experiment Station that has been immune from North American races of flax rust, and in Billings.

Except for the selection of Tammes Pale Blue, which had an occasional offtype plant, the differential varieties were pure for rust reaction when tested with the 24 races that had been identified from North American and South American collections of the pathogen. However, tests with F_2 cultures of hybrids of North American race 24 with South American race 22 showed that Argentine selection (C. I. 462) was homozygous for 1 pair of factors conditioning immunity from the known North American races and susceptibility to South American races 19, 20, and 22 but probably heterozygous for 2 other pairs of factors. Extensive tests with the F_2 hybrid rust cultures indicated that Burnham's selection of Morye (Argentine, C. I. 112) (*1*) was

[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

homozygous for these 3 pairs of rust-conditioning factors. Consequently, Morye has been substituted for C. I. 462 as a rust differential.

Data presented in this paper are the result of tests of the F_2 populations. Flax is adapted for such a procedure; under favorable conditions each plant produces several hundred seeds so that statistically significant results were usually obtained from the progeny of a single F_1 plant. Most of the rust-differentiating varieties of flax have sharp differences in reaction to the various physiologic races of the pathogen, being highly susceptible, highly resistant, or immune. This reduced the errors in reading the reaction of individual F_2 plants attributable to the effects that changes in environment exert on the expression of the intermediate infection types. Finally, the reaction of most of the differential varieties of flax to rust is inherited in a relatively simple manner, being conditioned by one, two, or three pairs of major factors.

These tests were conducted in the pathology greenhouse at the North Dakota Agricultural Experiment Station, Fargo, N. Dak., during the late fall, winter, and early spring. The usual test of 192 F_2 seeds gave sufficient plants for a statistical analysis of the results in crosses involving as many as 3 pairs of rust-conditioning factors. Eight seeds were sown in each 4½-inch pot in Fargo clay topsoil that had been steamed for 2 hours at 15 pounds' pressure. Because flax grows by elongation of the terminal bud it was possible to test each plant with as many as 5 physiologic races. This was done by inoculating the unfolding leaves of the terminal bud with 1 race when the seedlings were 2 to 3 inches tall and repeating the inoculation with additional races at 7- or 8-day intervals. As soon as the rust had developed sufficiently to differentiate the type of pustule, usually 9 to 10 days after inoculation, the type was recorded and the plant tagged with colored yarn representing a definite reaction to a specific race. The infected leaves were then picked off, leaving the terminal bud and apical leaves bearing the subsequent inoculation. When races having the required pathogenic capacities were available, the usual procedure was to inoculate the F_2 plants first with a race to which both parents were resistant, then with a race to which one parent was resistant and the other susceptible, and finally with a race to which the parents had the reverse reactions.

Plants were classified according to rust reaction on the basis of pustule type as previously described (*2*, *4*).

Immunity has invariably been dominant to resistance, and high resistance has usually been dominant to low. In some varieties the dominance of resistance is incomplete, plants heterozygous for a pair of rust-conditioning factors being less resistant to certain races than homozygous plants (*4*).

In the earlier studies the only races of rust used had been isolated from field collections. Consequently, in a number of crosses races capable of attacking one differential parent of the hybrid but unable to attack the other were not available. As a result of studies on the inheritance of pathogenicity in crosses between races 6 and 22 and races 22 and 24 (*6*), races of hybrid origin having almost all possible combinations of virulent and avirulent factors were obtained and used in the later studies.

TABLE 1.—*Inheritance of factors for rust reaction in flax varieties as determined by the reaction of the F_2 progeny to races of Melampsora lini, to which one parent was resistant or immune and the other susceptible*

C. I. No.	Differential parent and reaction to indicated race			Susceptible parent	F_2 plants having indicated reaction [1]			Pairs of factors	Theoretical ratio	χ^2 value	*P* between—
	Parent	Reaction [1]	Race		I	R	S				
					Number	*Number*	*Number*	*Number*			
355	Ottawa 770B	I	16	Bison	95	0	33	1	3:1	0.035	0.80 and 0.90.
188	Newland	I	21	Winona	137	0	46	1	3:1	.019	Do.
42	Bombay	I	1	Bison	151	0	38	1	3:1	2.405	0.10 and 0.20.
803–1	Williston Brown	R	52	do	0	143	45	1	3:1	.114	0.70 and 0.80.
515–1	Akmolinsk	R	1	do	0	144	48	1	3:1	.000	[2].
708–1	J. W. S.	I	1	do	119	0	43	1	3:1	.547	0.30 and 0.50.
647	Pale Blue Crimped	R—	24	do	0	[3] 100	26	1	3:1	1.280	0.20 and 0.30.
709–1	Kenya	R	52	do	0	[3] 136	56	1	3:1	1.777	0.10 and 0.20.
701	Abyssinian	I	1	do	149	0	43	1	3:1	.695	0.30 and 0.50.
836	Leona	R	85	Newland	0	149	43	1	3:1	.695	Do.
333–1	Tammes Pale Blue	I	9	J. W. S.	131	0	33	1	3:1	2.081	0.10 and 0.20.
184	Billings	I	24	Bison	139	0	41	1	3:1	.473	0.30 and 0.50.
270–1	Buda	I	7	do	234	57	19	2	12:3:1	.010	0.90 and 0.95.
25–1	Williston Golden	R	7	do	0	146	10	2	15:1	.063	0.80 and 0.90.
644	Bolley Golden	I	24	do	125	[3] 41	5	2	12:3:1	5.519	0.05 and 0.10.
1005–2	Italia Roma	I	24	do	140	[3] 38	14	2	12:3:1	.555	0.30 and 0.50.
438	Minn. 25–107	R	24	do	0	[3] 297	23	[4] 2	15:1	.480	Do.
416–3	Pale Verbena	I	4	do	[5] 178	0	7	[4] 2	15:1	1.920	0.10 and 0.20.
112	Morye	I	24	do	375	0	8	3	63:1	.668	0.30 and 0.50.
280	Rio	I	21	do	390	0	6	3	63:1	.360	0.50 and 0.70.
280	do	I	21	J. W. S.	205	0	3	3	63:1	.022	0.80 and 0.90.

[1] I, immune; R, resistant; R—, moderately resistant; S, susceptible.
[2] *P*, 1.00.
[3] Reaction of plants placed in this category somewhat variable, possibly because of the sensitivity of the intermediate infection types to differences in environment and to host vigor.
[4] These strains were not pure; some plants had only 1 pair of rust-conditioning factors.
[5] Some resistant plants included.

EXPERIMENTAL RESULTS

CROSSES BETWEEN DIFFERENTIAL RESISTANT AND CERTAIN SUSCEPTIBLE VARIETIES

Results obtained from tests to determine the number of factors for resistance to or immunity from rust possessed by each of the flax varieties studied are given in table 1. In these tests each F_2 progeny was inoculated with one or more races to which the differential parent was resistant and the other parent susceptible. These data show that resistance to or immunity from rust is dominant and conditioned by single pairs of factors in 12 of the varieties studied, by 2 factors in 6, and by 3 factors in 2.

It is possible that some of the differential flax varieties possess additional factors for rust resistance that were not effective against the races used in these studies. Consequently, the number of rust-conditioning factors possessed by each variety, as given in table 1, should be regarded as a minimum figure. Waterhouse and Watson (*12*), who found Bison to be immune from the races of flax rust occurring in their tests in Australia, used Bombay, a variety immune from South American and most North American races, as their susceptible host. Obviously, Bison would not have served as a susceptible parent if those particular Australian races had been used in the present studies.

Tests of F_2 of Bison × Buda and Bison × Williston Golden with various races (table 2) illustrate the need for determining the pathogenic

TABLE 2.—*Parental reaction to various races of Melampsora lini and segregation for reaction in F_2 plants of crosses of Bison × Buda and Bison × Williston Golden*

Cross and race tested	Reaction [1] to indicated race of—						χ^2 value	*P* between—
	Parent listed first	Parent listed second	F_2 plants					
Bison × Buda:								
7	S	I	I	I	R	S		
5	S	R	R	R	R−	S		
3	S	SR	SR	S− to S	SR	S		
20	S	I	I	I	S	S		
Plants observed			188	46	57	19	3.622	0.30 and 0.50.
Plants expected (9:3:3:1)			175	58	58	19		
Bison × Williston Golden:								
7	S	R	R	R	R	S		
52	S	R	R	R	S	S		
16	S	R	R	S	R	S		
Plants observed			92	26	28	10	.625	0.80 and 0.90.
Plants expected (9:3:3:1)			88	29	29	10		

[1] I, immune; R, resistant; R−, moderately resistant; SR, semiresistant; S−, moderately susceptible; S, susceptible.

capacities of the races used as well as for reserving judgment as to the finality of the results. The segregation of F_2 plants of Bison × Buda for reaction to the races tested can be explained by assuming that immunity from or resistance to rust was dominant and that Bison carried no rust-conditioning factors and Buda two pairs. Both pairs were effective in conditioning resistance to race 5; one was almost inoperative against race 3, but conditioned immunity from races 7 and

20; the second pair was inoperative against race 20, but conditioned semiresistance to race 3 and resistance to race 7.

Segregation for rust reaction in the F_2 population of Bison × Williston Golden to races 7, 52, and 16 of *Melampsora lini* illustrates the specific relation that exists between the different races of the pathogen and the individual factors conditioning rust resistance in the host. The parent varieties reacted the same to each of these 3 races, Bison being susceptible and Williston Golden resistant. The segregation of F_2 plants for reaction to race 7 of 146 resistant to 10 susceptible approximated a 15:1 ratio, suggesting that resistance to race 7 in Williston Golden was conditioned by 2 pairs of duplicate factors. The 10 F_2 plants susceptible to race 7 were susceptible also to races 52 and 16. Of the 146 F_2 plants resistant to race 7, 92 were also resistant to races 16 and 52, 28 were resistant to race 16 and susceptible to race 52, and 26 were resistant to race 52 and susceptible to race 16. This ratio of segregates for reaction to each race approximates that expected if rust resistance is a dominant character and Williston Golden possesses 2 pairs of rust-conditioning factors, of which one is operative against race 52 and the other against race 16 and both condition resistance to race 7.

CROSSES BETWEEN DIFFERENTIAL VARIETIES

As the study progressed, it became apparent that most of the factors for rust reaction lay in the three allelomorphic series or linkage groups represented by the factors occurring in Ottawa 770B, Newland, and Bombay. Rust reaction in each of these varieties apparently is conditioned by a single pair of independently inherited factors. Ottawa 770B, Newland, and Bombay have been either immune from or highly susceptible to every physiologic race with which they have been tested, suggesting that secondary rust-conditioning factors are absent. In later phases of these investigations inheritance of rust reaction in the F_2 population of hybrids of the variety being studied with each of these three varieties was first determined. The lack of segregates susceptible to races to which both parents were immune or resistant was interpreted as indicating the presence of a rust-conditioning factor in the variety being studied which was allelomorphic to or linked with the factor for immunity in Ottawa 770B, Newland, or Bombay as the case might be. The occurrence of appropriate numbers of susceptible segregates indicated lack of allelomorphism or linkage.

CROSSES INVOLVING OTTAWA 770B

Of the 11 differential varieties in which rust reaction is conditioned by a single pair of factors, 4 have factors that lie in the Ottawa 770B allelomorphic series (table 3). These varieties, in addition to Ottawa 770B, are J. W. S., Pale Blue Crimped, and Kenya. One of the 2 pairs of rust-conditioning factors in Buda is allelomorphic to the Ottawa 770B factor, as is 1 of the 2 in Williston Golden. Both pairs of rust-conditioning factors possessed by Bolley Golden and Italia Roma are inherited independently of the pair in Ottawa 770B.

None of the 754 F_2 plants of the cross Morye × Ottawa 770B was susceptible to race 24, which attacked neither parent; this indicates an allelic relation between 1 of the 3 rust-conditioning factors in Morye and that in Ottawa 770B. Since some plants were susceptible to both

TABLE 3.—*Parental reaction to various races of Melampsora lini and segregation of F_2 plants of crosses involving Ottawa 770B*

Cross and race tested	Reaction [1] to indicated race of—						χ^2 value	P between—
	Parent listed first	Parent listed second	F_2 plants					
Ottawa 770B × Newland:								
7	I	I	I	I	S			
20	I	S	I	S	S			
Plants observed			147	34	11		0.257	0.80 and 0.90.
Plants expected (12:3:1)			144	36	12			
Ottawa 770B × Bombay:								
3	I	I	I	I	I	S		
22	S	I	I	I	S	S		
24	I	S	I	S	I	S		
Plants observed			110	43	32	9	2.563	0.30 and 0.50.
Plants expected (9:3:3:1)			109	36	36	12		
Williston Brown × Ottawa 770B:								
52	R	I	I	I	R	S		
7	R	I	I	I	R	S		
47	R	S	R	S	R	S		
16	S	I	I	I	S	S		
Plants observed			103	35	39	15	1.259	0.70 and 0.80.
Plants expected (9:3:3:1)			108	36	36	12		
Ottawa 770B × Akmolinsk:								
2	I	R	I	R	S			
7	I	S	I	S	S			
Plants observed			101	22	5		1.552	0.30 and 0.50.
Plants expected (12:3:1)			96	24	8			
Ottawa 770B × J. W. S.:								
19	I	I	I	I	I	S		
7	I	S	I	I	S	S		
22	S	I	I	S	I	S		
Plants observed			95	45	48	0	.117	0.90 and 0.95.
Plants expected (2:1:1:0)			94	47	47	0		
Ottawa 770B × Pale Blue Crimped:								
7	I	R—	R and I	S				
Plants observed			124	0			(?)	(?).
Plants expected (1:0)			124	0				
Kenya × Ottawa 770B:								
7	R	I	I	I	I	S		
68	R	S	R	R	S	S		
79	S	I	I	S	I	S		
Plants observed			35	13	16	0	.844	0.50 and 0.70.
Plants expected (2:1:1:0)			32	16	16	0		
Ottawa 770B × Abyssinian:								
1	I	I	I	I	S			
3	I	R	I	R	S			
8	I	S	I	S	S			
Plants observed			137	45	18		5.047	0.05 and 0.10.
Plants expected (12:3:1)			150	38	12			
Leona × Ottawa 770B:								
24	R	I	I	R	I	S		
68	R	S	R	R	S	S		
52	S	I	I	S	I	S		
Plants observed			113	38	27	6	5.487	0.10 and 0.20.
Plants expected (9:3:3:1)			104	34	34	12		
Ottawa 770B × Tammes Pale Blue:								
16	I	I	I	I	S			
20	I	S	I	S	S			

See footnotes at end of table.

TABLE 3.—*Parental reaction to various races of Melampsora lini and segregation of F_2 plants of crosses involving Ottawa 770B*—Continued

Cross and race tested	Reaction [1] to indicated race of—							χ^2 value	*P* between—
	Parent listed first	Parent listed second	F_2 plants						
Plants observed			146	30	14			1.356	0.50 and 0.70.
Plants expected (12:3:1).			142	36	12				
Ottawa 770B × Buda:									
20	I	I	I	I	I	S			
3	I	SR	I	R to SR	S	S			
21	I	S	I	S	S	S			
Plants observed			148	35	14	0		.333	0.80 and 0.90.
Plants expected (12:3:1:0).			148	37	12	0			
Williston Golden × Ottawa 770B:									
52	R	I	I	I	R	S	S		
47	R	S	R	S	R	S	S		
16	R	I	I	I	R	R	S		
24	S	I	I	I	S	S	S		
Plants observed			112	37	31	12	0	.870	0.80 and 0.90.
Plants expected (9:3:3:1:0).			108	36	36	12	0		
Ottawa 770B × Bolley Golden:									
16	I	I	I	I	R	S			
19	I	S	I	S	S	S			
Plants observed			237	49	19	4		3.047	0.30 and 0.50.
Plants expected (48:12:3:1).			232	58	14	5			
Italia Roma × Ottawa 770B:									
24	I	I	I	I	I	I	R S		
68	I	S	I	I	R− to S−	S	R S		
105	S	I	I	S	I	I	S S		
Plants observed			98	31	24	12	10 3	2.210	0.80 and 0.90.
Plants expected (36:12:9:3:3:1).			100	33	25	8	8 3		
Morye × Ottawa 770B:									
24	I	I	I	I	I	I	S		
68	I	S	I	I	S	S	S		
75	S−	I	I	S	I	S	S		
Plants observed			485	183	70	16	0	39.375	(3).
Plants expected (45:15:3:1:0).			530	177	35	12	0		
Billings × Ottawa 770B:									
24	I	I	I	I	I	S			
68	I	S	I	I	S	S			
85	S	I	I	S	I	S			
Plants observed			114	33	28	7		4.138	0.20 and 0.30.
Plants expected (9:3:3:1).			103	34	34	11			
C. I. 438 × Ottawa 770B:									
7	R	I	I	I	S				
1	R−	I	I	R−	S				
20	S	I	I	S	S				
Plants observed			137	45	0			.007	0.90 and 0.95.
Plants expected (3:1:0).			136	46	0				
C. I. 416-3 × Ottawa 770B:									
41	I	I	I	I	S				
20	S	I	I	S	S				
Plants observed			268	76	25			1.176	0.50 and 0.70.
Plants expected (12:3:1).			277	69	23				
Ottawa 770B × Rio:									
16	I	I	I	I	R	S			
20	I	S	I	S	S	S			
Plants observed			223	64	5	0		.403	0.80 and 0.90.
Plants expected (48:15:1:0).			219	68	5	0			

[1] I, immune; R, resistant; R−, moderately resistant; SR, semiresistant; S−, moderately susceptible; S, susceptible.

[2] No segregation to races tested.

[3] $P, <0.01$.

race 68 and race 75 it must be assumed that the allelomorphic factors are inoperative against race 68. However, the occurrence of 70 segregates immune from races 24 and 75 and susceptible to race 68 cannot be satisfactorily explained by assuming that immunity from the last-named race is conditioned by either or both of the nonallelomorphic rust-conditioning factors in Morye. This number of segregates approximates that expected if race 68 were a mixture of 2 races, one of which was virulent on Ottawa 770B and on plants carrrying the rust-conditioning factor in Morye allelic to that in Ottawa 770B and the other virulent on Ottawa 770B and on plants carrying one of the nonallelic rust-conditioning factors possessed by the Morye parent. However, methods usually employed successfully in isolating the components of race mixtures have failed to indicate that race 68 is not pure.

The C. I. 438 parent was heterozygous; in some crosses it contributed two rust-conditioning factors and in others one. In the cross C. I. 438 × Ottawa 770B the single factors in each variety were allelic. The C. I. 438 factor is probably distinct from those possessed by any of the differential varieties.

One of the three factors in Rio also lies in the Ottawa 770B allelomorphic series. Although this factor in Rio may be distinct, at the present time there is no experimental basis for separating it from the Morye factor.

CROSSES INVOLVING NEWLAND

In every cross involving Newland (table 4) the χ^2 value for fit of the observed to the expected ratio of segregates gave a P of more than 0.10, indicating agreement of the data with the hypotheses and regularity in segregation of the Newland factor. The observed ratios may be explained by assuming that the single pair of rust-conditioning factors in Williston Brown and one of the two in Buda, Williston Golden, Bolley Golden, and Italia Roma are allelic to the pair in Newland. The rust-conditioning factors in the other differential varieties, including all three pairs in Morye, are inherited independently of the Newland factor. The single pair in Billings is allelic to that in Newland and one of the three in Rio may be, but the number of plants studied was not sufficient to give statistically significant results in a hybrid involving four factors.

Myers (*8*) concluded that a near-immune factor allelic to that in Newland conditioned reaction in C. I. 416–3 to the races he used. The parent material used in the present study apparently was heterozygous, since the ratios of F_2 segregates in C. I. 416–3 × Bison (table 1) and C. I. 416–3 × Bombay (see table 5) may be explained by assuming that C. I. 416–3 carried two rust-conditioning factors, whereas the segregation ratios of the F_2 hybrids of C. I. 416–3 with Ottawa 770B (table 3) and Newland (table 4) indicate that each parent contributed but a single rust-conditioning factor. In the cross C. I. 416–3 × Newland, no segregates susceptible to race 16, from which both parents are immune, were obtained; this substantiated Myers' conclusions that in these varieties rust-conditioning factors are allelic. The F_2 plants segregated into a ratio approximating 3 immune to 1 not immune, the latter ranging in reaction from necrosis of the inoculated tissue to moderate susceptibility. Since the Newland factor has invariably conditioned immunity from race 16, it is probable that modifying factors

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TABLE 4.—*Parental reaction to various races of Melampsora lini and segregation of F_2 plants of crosses involving Newland*

Cross and race tested	Reaction [1] to indicated race of—						χ^2 value	*P* between—
	Parent listed first	Parent listed second	F_2 plants					
Ottawa 770B × Newland	(2)	(2)	(2)				(2)	(3).
Bombay × Newland:								
1	I	I	I	I	I	S		
19	I	S	I	I	S	S		
24	S	I	I	S	I	S		
Plants observed			104	36	40	12	0.592	0.80 and 0.90.
Plants expected (9:3:3:1).			108	36	36	12		
Williston Brown × Newland:								
7	R	I	I	I	R	S		
47	R	S	R	S	R	S		
16	S	I	I	I	S	S		
Plants observed			83	38	38	0	.308	0.80 and 0.90.
Plants expected (2:1:1:0).			79	40	40	0		
J. W. S. × Newland:								
1	I	I	I	I	I	S		
9	S	I	I	I	S	S		
19	I	S	I	S	I	S		
Plants observed			104	35	43	10	1.780	0.50 and 0.70.
Plants expected (9:3:3:1).			108	36	36	12		
Kenya × Newland:								
24	R	I	I	I	R	S− to S		
81	S	I	I	I	R− to S	S− to S		
73	R	S	R	S−	R	S− to S		
Plants observed			113	32	32	7	2.995	0.30 and 0.50.
Plants expected (9:3:3:1).			104	34	34	12		
Newland × Leona:								
24	I	R	I	I	R	S		
52	I	S	I	I	S	S		
85	S	R	I	S	R	S		
Plants observed			114	26	35	17	5.222	0.10 and 0.20.
Plants expected (9:3:3:1).			108	36	36	12		
Newland × Tammes Pale Blue:								
1	I	I	I	S				
Plants observed			175	7			1.794	0.10 and 0.20.
Plants expected (15:1).			171	11				
Buda × Newland:								
7	I	I	I	I to R	I	S		
3	SR	I	I	R to SR	I	S		
4	S	I	I	S	I	S		
111	R	S	R	R	S	S		
Plants observed			158	51	13	0	.609	0.70 and 0.80.
Plants expected (11:4:1:0).			153	55	14	0		
Williston Golden × Newland:								
7	R	I	I	R	I	R S		
16	R	I	I	R	I	S S		
52	R	I	I	R	I	R S		
47	R	S	R−	R	S	R S		
68	S	I	I	S	I	S S		

See footnotes at end of table.

TABLE 4.—*Parental reaction to various races of Melampsora lini and segregation of F_2 plants of crosses involving Newland*—Continued

Cross and race tested	Reaction [1] to indicated race of—							χ^2 value	P between—
	Parent listed first	Parent listed second	F_2 plants						
Plants observed			95	28	14	13	0	4.325	0.20 and 0.30.
Plants expected (11:3:1:1:0).			103	28	9	9	0		
Newland × Bolley Golden:									
16	I	I	I	S					
41	I	I	I	S					
Plants observed			192	0				[2]	[3].
Plants expected (1:0).			192	0					
Italia Roma × Newland:									
24	I	I	I	I	I	I	S		
60	I	S	I	I	R−	S	S		
52A	R−	I	I	R− to S	I	I	S		
Plants observed			101	48	32	10	0	1.156	0.70 and 0.80.
Plants expected (8:4:3:1:0).			95	48	36	12	0		
Morye × Newland:									
24	I	I	I	I	I	S			
60	I	S	I	I	S	S			
52	I	I	I	S	I	S			
Plants observed			173	15	2	2		3.488	0.30 and 0.50.
Plants expected (237:15:3:1).			178	11	2	1			
Billings × Newland:									
24	I	I	I	I	S				
47	I	S	I	S	S				
Plants observed			143	42	0			.521	0.30 and 0.50.
Plants expected (3:1:0).			139	46	0				
C. I. 438 × Newland:									
16	R−	I	I	R−	S				
Plants observed			140	51	1			2.244	0.30 and 0.50.
Plants expected (48:15:1).			144	45	3				
C. I. 416-3 × Newland:									
16	I	I	I	I [4] to S−	S				
Plants observed			142	39	0			1.151	0.20 and 0.30.
Plants expected (3:1:0).			136	45	0				
Rio × Newland:									
1	I	I	I	S					
16	I	I	I	S					
41	I	I	I	S					
Plants observed			384	0				[2]	[3].
Plants expected (1:0).			384	0					

[1] I, immune; R, resistant; R−, moderately resistant; SR, semiresistant; S−, moderately susceptible; S, susceptible.
[2] See table 3.
[3] No segregation to races tested.
[4] Plants classed as immune showed severe necrosis and stunting.

were present in C. I. 416–3 or that environment exerts a considerable influence on the effectiveness of the rust-conditioning factors possessed by this variety.

The rust-conditioning factor in Williston Brown and the factor in Williston Golden allelic to that in Newland are probably identical,

since Williston Brown originated as a brown-seeded selection from a lot of Williston Golden and has been susceptible to all races attacking that variety (*3*, *6*).

It has not been established whether the factors in Bolley Golden and Italia Roma which are allelic to the Newland factor and condition immunity from North American races of rust are identical or allelic. Since the data on the inheritance of rust-conditioning factors in these varieties may be explained by assuming that these factors are identical, they will be so considered until contrary evidence is obtained. Thus, although six differential varieties have factors in the Newland allelomorphic series, the assumption of only four separate factors satisfactorily explains the current data.

Crosses of Newland with three of the differential varieties, Akmolinsk, Abyssinian, and Pale Blue Crimped, were not studied. However, tests indicated that rust reaction in each of these varieties is conditioned by single factors, that of Pale Blue Crimped being allelic to the Ottawa 770B factor and those of Akmolinsk and Abyssinian being linked with the Bombay factor.

CROSSES INVOLVING BOMBAY AND PUNJAB

Rust reaction in Bombay and Punjab apparently is conditioned by a common pair of factors as these varieties have given identical reactions to all races with which they have been tested. Bombay and Punjab have been used interchangeably as parents in studying the interaction of the Bombay factor with the rust-resistant factors of other varieties (table 5).

In crosses of Bombay with varieties having single rust-conditioning factors allelic to those in Ottawa 770B (J. W. S., Pale Blue Crimped, and Kenya) or Newland (Williston Brown, Billings, and C. I. 416–3), the F_2 populations segregated for reaction to selected races of *Melampsora lini* in accordance with the simple Mendelian ratios expected if the factors are independently inherited. Likewise, in the crosses of Punjab or Bombay with Buda and Williston Golden, each of which has rust-conditioning factors in both the Ottawa 770B and the Newland series, the observed ratios of segregates satisfactorily approached those expected if all 3 rust-conditioning factors involved in these crosses are independently inherited. Since 1 of the 2 factors conditioning rust reaction in Bolley Golden is allelic to that in Newland, the occurrence of segregates susceptible to race 16 from which both parents were immune indicates that the second rust factor in Bolley Golden is inherited independently of that in Bombay. Insufficient seeds to make a statistically significant analysis for the segregation of rust-conditioning factors were available in the cross Italia Roma × Bombay, and the data are inconclusive. However, the assumption that the rust-conditioning factors in this cross are independently inherited gives a poorer fit of the observed to the expected ratio of segregates than does the assumption that 1 of the rust-conditioning factors in Italia Roma is linked with the Bombay factor and that crossing over accounts for the 1 of 48 F_2 plants being susceptible to the race attacking neither parent.

Tests on the inheritance of rust reaction in the two progenies of the cross C. I. 438 × Bombay indicate that the C. I. 438 parent was heterozygous. The ratio of segregates for reaction to race 5 may be ex-

TABLE 5.—*Parental reaction to various races of Melampsora lini and segregation of F_2 plants of crosses involving Bombay or Punjab*

Cross and race tested	Reaction [1] to indicated races of—							χ^2 value	*P* between—
	Parent listed first	Parent listed second	F_2 plants						
Ottawa 770B × Bombay	(2)	(2)	(2)					(2)	(2).
Bombay × Newland	(3)	(3)	(3)					(3)	(3).
Williston Brown × Bombay:									
52	R	I	I	I	R	S			
55	R	S	R	S	R	S			
16	S	I	I	I	S	S			
Plants observed			93	35	45	11		4.289	0.20 and 0.30.
Plants expected (9:3:3:1).			104	34	34	12			
Bombay × Akmolinsk:									
1	I	R	I	I	R	S			
5	I	S	I	I	S	S			
41	S	R	R	S	R	S			
Plants observed			72	44	43	1		1.425	0.70 and 0.50.
Plants expected (2:1:1:0).			80	40	40	0			
Bombay × J. W. S.:									
1	I	I	I	I	I	S			
41	S	I	I	I	S	S			
16	I	S	I	S	I	S			
Plants observed			117	36	27	12		3.000	0.30 and 0.50.
Plants expected (9:3:3:1).			108	36	36	12			
Pale Blue Crimped × Bombay:									
7	R—	I	I and R	S					
Plants observed			99	11				2.640	0.10 and 0.20.
Plants expected (15:1).			103	7					
Kenya × Bombay:									
6	R	I	I	I	I	S			
24	R	S	I	I	S	S			
79	S	I	I	S	I	S			
Plants observed			31	16	14	3		1.250	0.50 and 0.70.
Plants expected (9:3:3:1).			36	12	12	4			
Bombay × Abyssinian:									
7	I	R	I	R	S				
Plants observed			51	13	0			.750	0.30 and 0.50.
Plants expected (3:1:0).			48	16	0				
Leona × Bombay:									
6	R	I	I	I	R	S			
52	S	I	I	I	S	S			
73	R	S	I	S	R	S			
Plants observed			85	39	64	2		8.306	0.01 and 0.02.
Plants expected (2:1:1:0).			94	48	48	0			
Tammes Pale Blue × Punjab:									
2	I	I	I	I	I	S			
41	I	S	I	I	S	S			
19	S	I	I	S	I	S			
Plants observed			114	48	30	0		10.125	(4).
Plants expected (2:1:1:0).			96	48	48	0			
Punjab × Buda:									
5	I	R	I	I	R	R	S		
3	I	SR	I	I	SR	S	S		
16	I	S	I	I	S	S	S		
41	S	SR to S—	S—	S	S—	S	S		

See footnotes at end of table.

TABLE 5.—*Parental reaction to various races of Melampsora lini and segregation of F_2 plants of crosses involving Bombay or Punjab*—Continued

Cross and race tested	Reaction [1] to indicated races of—							χ^2 value	*P* between—
	Parent listed first	Parent listed second	F_2 plants						
Plants observed	.....	.....	122	31	27	10	2	5.103	0.20 and 0.30.
Plants expected (36:12:12:3:1).	.....	.....	108	36	36	9	3		
Bombay × Williston Golden:									
1	I	R	I	I	R	S		.....	.....
2	I	S	I	I	S	S		.....	.....
41	S	R	R	S	R	S		.....	.....
Plants observed	.....	.....	99	31	45	13		3.772	0.20 and 0.30.
Plants expected (9:3:3:1).	.....	.....	106	35	35	12			
Bolley Golden × Punjab:									
16	I	I	I	I	I	I	R− S	.....	.....
41	I	S	I	I	R−	S	R− S	.....	.....
20	S	I	I	S	I	I	S S	.....	.....
Plants observed	.....	.....	300	119	70	21	31 9	5.333	0.30 and 0.50.
Plants expected (36:12:9:3:3:1).	.....	.....	309	103	77	26	26 9		
Italia Roma × Bombay:									
6	I	I	I	I	I	R to S− S		.....	.....
24	I	S	R to I	I	S	S− to S S		.....	.....
105	S	I	I	S	I	S	S	.....	.....
Plants observed	.....	.....	28	12	1	6	1	10.558	0.02 and 0.05.
Plants expected (45:12:3:3:1).[5]	.....	.....	34	9	2	2	1		
Plants expected (11:3:1:1:0).[6]	.....	.....	33	9	3	3	0	6.226	0.10 and 0.20.
Bombay × Billings:									
1	I	I	I	I	I	S		.....	.....
24	S	I	I	S	I	S		.....	.....
85	I	S	I	I	S	S		.....	.....
Plants observed	.....	.....	138	30	28	11		9.296	0.02 and 0.05.
Plants expected (9:3:3:1).	.....	.....	116	39	39	13			
C. I. 438 × Bombay:									
5	R	I	I	R to S− S				.....	.....
Plants observed	.....	.....	130	37	5			3.782	0.10 and 0.20.
Plants expected (48:15:1).	.....	.....	129	40	3				
C. I. 438 × Bombay:									
5	R	I	I	R to S− S				.....	.....
Plants observed	.....	.....	134	41	13			1.419	0.30 and 0.50.
Plants expected (12:3:1).	.....	.....	141	35	12				
C. I. 416-3 × Bombay:									
16	I	I	I	I	S			.....	.....
24	I	S	I	S− to S S				.....	.....
Plants observed	.....	.....	147	40	1			1.908	0.30 and 0.50.
Plants expected (48:15:1).	.....	.....	141	44	3				
Rio × Bombay:									
16	I	I	I	I	I	I	S	.....	.....
41	I	S	I	I	R to S−	S	S	.....	.....
19	S	I	I	S	I	I	S	.....	.....
Plants observed	.....	.....	101	77	11	3	0	25.165	(4).
Plants expected (44:16:3:1:0).	.....	.....	132	48	9	3	0		

[1] I, immune; R, resistant; R−, moderately resistant; SR, semiresistant; S−, moderately susceptible; S, susceptible. [2] See table 3. [3] See table 4. [4] P, <0.01.
[5] Each of 3 pairs of rust-conditioning factors assumed to be independently inherited.
[6] 2 of the 3 pairs of rust-conditioning factors assumed to be allelic.

plained by assuming that in one progeny the C. I. 438 parent contributed two pairs of independently inherited rust-conditioning factors and the Bombay parent one, whereas in the second progeny the assumption that each parent contributed a single pair explains the observed ratio of segregates.

In contrast with the regular segregation and absence of crossing over in hybrids of varieties having factors in the Ottawa 770B or the Newland series, crosses of Bombay or Punjab with varieties having rust-conditioning factors in the same linkage group are marked by irregular ratios and crossing over. Of crosses of varieties having rust-conditioning factors in the Bombay linkage group only the cross Bombay × Abyssinian is entirely regular, but only one-third the usual number of plants was tested. The observed ratio of segregates of the cross Bombay × Akmolinsk approximates that expected except that 1 of 160 plants was susceptible to race 1, which attacked neither parent. This indicates that the rust-conditioning factors in these varieties are linked rather than allelic. Crossing over was also evident in the cross Leona × Bombay, but even if this is disregarded, the observed ratio of segregates deviates significantly from that expected if simple Mendelian inheritance governs the transmission of rust-conditioning factors. In Tammes Pale Blue × Punjab no crossing over was observed, but the observed ratio of segregates differs significantly from that expected if the single rust-conditioning factor in each parent is allelic. In the cross Rio × Bombay, in which 4 rust-conditioning factors are involved, an insufficient number of plants was studied for a significant statistical analysis, but the deviation of the observed from the theoretical ratio suggests that the Bombay factor for immunity from race 19 is linked with 1 of the 3 Rio factors for immunity from or resistance to race 16. Sufficient F_2 seeds of Bombay × Morye were not available for study.

CROSSES OF ADDITIONAL DIFFERENTIAL VARIETIES

Some crosses between differential varieties not involving Ottawa 770B, Newland, and Bombay or Punjab (table 6) were studied before it had been determined that most rust-conditioning factors in flax lie in the allelomorphic series or linkage groups represented by the factors in the above-named varieties. Other crosses were studied later to verify the allelic or linkage relations as determined in the tests of hybrids involving Ottawa 770B, Newland, aid Bombay and to demonstrate the effectiveness of selected races of the pathogen in resolving the F_2 populations into their phenotypic components.

Without exception the data (table 6) substantiate the conclusions regarding interaction of the rust-conditioning factors in the differential varieties based on the study of crosses with Ottawa 770B, Newland, and Bombay or Punjab. No F_2 plant susceptible to races attacking neither parent was observed in the crosses Williston Golden × J. W. S., Morye × J. W. S., Rio × J. W. S., and Pale Blue Crimped × Buda, hybrids in which each parent has a rust-conditioning factor allelic to that in Ottawa 770B. Likewise, in the crosses Williston Brown × Williston Golden and Bolley Golden × Williston Golden, varieties having a factor for rust reaction allelic to that in Newland, and in the cross Akmolinsk × Abyssinian, each of which has a rust-conditioning factor linked with that in Bombay, no F_2 segregate sus-

TABLE 6.—*Parental reaction to various races of Melampsora lini and segregation of F_2 plants of crosses not involving Ottawa 770B, Newland, and Bombay or Punjab*

Cross and race tested	Reaction [1] to indicated race of—			χ^2 value	*P* between—
	Parent listed first	Parent listed second	F_2 plants		
Williston Brown × J. W. S.:					
52	R	I	I I R S		
7	R	S	R S R S		
24	S	I	I I S S		
Plants observed			116 33 32 11	1.369	0.50 and 0.70.
Plants expected (9:3:3:1).			108 36 36 12		
Pale Blue Crimped × Williston Brown:					
7	R−	R	R S		
Plants observed			120 8	0.000	[2].
Plants expected (15:1).			120 8		
Abyssinian × Williston Brown:					
7	R	R	R S		
Plants observed			58 6	1.067	0.30 and 0.50.
Plants expected (15:1).			60 4		
Williston Brown × Williston Golden:					
7	R	R	R S		
52	R	R	R S		
Plants observed			64 0	[3]	[3].
Plants expected (1:0).			64 0		
Akmolinsk × J. W. S.:					
1	R	I	I I R S		
5	S	I	I I S S		
9	R	S	I S R S		
Plants observed			112 32 40 8	2.369	0.30 and 0.50.
Plants expected (9:3:3:1).			108 36 36 12		
Akmolinsk × Abyssinian:					
1	R	I	I R S		
3	S	R	R S S		
Plants observed			130 38 0	.508	0.30 and 0.50.
Plants expected (3:1:0).			126 42 0		
Buda × Akmolinsk:					
7	I	S	I and R I and R I I S S		
3	SR	S	SR SR S S S S		
9	S−	R	R S− R S		
4	S	R	R S R S R S		
Plants observed			63 24 13 5 3 1	1.984	0.80 and 0.90.
Plants expected (36:12:9:3:3:1).			62 20 15 5 5 2		
Williston Golden × Akmolinsk:					
1	R	R	R R R S R S		
7	R	S	R R R R S S		
2	S	R	R S R S R S		
5	R	S	R R S S S S		
Plants observed			113 32 27 13 5 2	4.341	0.50 and 0.70.
Plants expected (36:12:9:3:3:1).			108 36 27 9 9 3		

See footnotes at end of table.

TABLE 6.—*Parental reaction to various races of Melampsora lini and segregation of F_2 plants of crosses not involving Ottawa 770B, Newland, and Bombay or Punjab*—Continued

Cross and race tested	Reaction [1] to indicated race of—										χ^2 value	P between—
	Parent listed first	Parent listed second	F₂ plants									
Williston Golden × J. W. S.:												
1	R	I	I	R	I	S						
7	R	S	R	R	S	S						
2	S	I	I	S	I	S						
Plants observed			138	39	15	0					2.710	0.20 and 0.30.
Plants expected (11:4:1:0).			132	48	12	0						
J. W. S. × Bolley Golden:												
24	I	I	I	I	I	I	R	S				
21	S	I	I	I	R	S	R	S				
19	I	S	I	S	I	I	S	S				
Plants observed			321	85	69	22	22	8			4.922	0.30 and 0.50.
Plants expected (36:12:9:3:3:1).			296	99	74	25	25	8				
Morye × J. W. S.:												
16	I	S	I	S	S							
24	I	I	I	I	S							
Plants observed			190	2	0						.338	0.50 and 0.70.
Plants expected (63:1:0).			189	3	0							
Rio × J. W. S.:												
7	I	S	I	I	I	S	S					
16	I	S	I	I	R	S	S					
20	S	I	I	S	I	I	S					
Plants observed			104	54	49	3	0				.081	0.99 and 1.00.
Plants expected (32:16:15:1:0).			105	53	49	3	0					
Pale Blue Crimped × Buda:												
7	R−	I	I	R	S							
Plants observed			91	25	0						.736	0.30 and 0.50.
Plants expected (3:1:0).			87	29	0							
Williston Golden × Abyssinian:												
47	R	I to R	R	R	R	R	R	R	S	S		
52	R	S	R	R	S	R	S	R	S	S		
16	R	S	R	R	R	S	S	S	R	S		
68	S	I to R	R	S	R	R	R	S	S	S		
Plants observed			72	19	24	21	12	6	5	5	6.946	0.30 and 0.50.
Plants expected (27:9:9:3:3:3:1).			69	23	23	23	8	8	8	3		
Bolley Golden × Williston Golden:												
7	I	R	I	I	I	I	I	S				
16	I	R	I	I	R	R	S	S				
52	I	R	I	I	R	R	R	S				
24	I	S	I	I	R	S	S	S				
73	R	S	R	S	R	S	S	S				
Plants observed			104	39	31	9	5	0			2.392	0.50 and 0.70.
Plants expected (36:12:12:3:1:0).			105	35	35	9	3	0				

[1] I, immune; R, resistant; R−, moderately resistant; SR, semiresistant; S−, moderately susceptible; S, susceptible.
[2] P, 1.00.
[3] No segregation to races tested.

ceptible to races attacking neither parent was observed. Conversely, in the crosses Williston Brown × J. W. S., Pale Blue Crimped × Williston Brown, Abyssinian × Williston Brown, Akmolinsk ×

J. W. S., Buda × Akmolinsk, Williston Golden × Akmolinsk, J. W. S. × Bolley Golden, and Williston Golden × Abyssinian, varieties in which the factors for rust reaction are not allelic or linked, the F_2 populations segregated for reaction to rust into ratios approximating those expected if the factors conditioning rust reaction are independently inherited.

Of special interest is the test of the cross Williston Golden × Abyssinian. Rust reaction in the former is conditioned by two factors, one allelic to that in Ottawa 770B and the other allelic to that in Newland. The single rust-conditioning factor in Abyssinian lies in the Bombay linkage group. By inoculating the F_2 plants successively with races 47, 52, 16, and 68, the eight phenotypic groups theoretically possible in such a trifactorial hybrid were differentiated and χ^2 for fit of the observed to the theoretical 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 ratio of segregates gave a P value lying between 0.30 and 0.50, indicating good agreement between the data and the hypothesis.

DISCUSSION AND CONCLUSIONS

Suggested genotypes of the 16 rust-differential flax varieties are given in table 7. These 16 varieties possess 22 pairs of rust-conditioning factors of which at least 19 are distinct. Other varieties have additional rust-conditioning factors distinct from those possessed by the 16 differentials.

Seven of the differential varieties have rust-conditioning factors in the *LL* series, 6 in the *MM* series, and 5 in the *NN* series. Each factor in the *LL* series appears to be distinct. Of the varieties having factors in the *MM* series Williston Brown and Williston Golden and Bolley Golden and Italia Roma appear to have common factors. The study on the inheritance of pathogenicity in flax rust (*6*) indicates that one of the unplaced factors in Morye may be identical with the

TABLE 7.—*Suggested genotypes of rust-differentiating and other varieties of flax*

Variety	C. I. No.	Pairs of factors for rust reaction	Suggested genotype		
Differential varieties:		*Number*			
Ottawa 770B	355	1	LL	mm	nn.
Newland	188	1	"	MM	nn.
Bombay	42	1	"	mm	NN.
Williston Brown	803–1	1	"	M^1M^1	nn.
Akmolinsk	515–1	1	"	mm	N^1N^1.
J. W. S.	708–1	1	L^2L^2	mm	nn.
Pale Blue Crimped	647	1	L^2L^2	mm	nn.
Kenya	709–1	1	L^4L^4	mm	nn.
Abyssinian	701	1	"	mm	N^2N^2.
Leona	836	1	"	mm	N^3N^3.
Tammes Pale Blue	333–1	1	"	mm	N^4N^4.
Buda	270–1	2	L^1L^1	M^2M^2	nn.
Williston Golden	25–1	2	L^5L^5	M^1M^1	nn.
Bolley Golden [1]	644	2	"	M^3M^3	nn.
Italia Roma	1005–2	2	"	M^3M^3	(2).
Morye [1]	112	3	L^6L^6	mm	(3).
Other varieties:					
Billings	184	1	"	M^4M^4	nn.
Minn. 25–107 [4]	438	1 and 2	L^7L^7	mm	nn.
Pale Verbena [4]	416–3	1 and 2	"	M^3M^5	nn.
Rio	280	3	L^6L^6	M^6M^6	N^5N^5.

[1] This variety has 1 factor lying outside the *L*, *M*, or *N* series.
[2] The location of this factor not definitely established but probably in the *N* series.
[3] Not tested.
[4] Not pure; some plants have 1 and others 2 resistant factors.

factors in the *NN* series conditioning rust reaction in Tammes Pale Blue. As there was no crossing over in hybrids between varieties having factors in either the *LL* series or the *MM* series, these factors are considered to be allelomorphic. Crossing over and irregular segregation occurred in most hybrids involving varieties having factors in the *NN* series; therefore these factors are considered to be linked rather than allelomorphic.

Studies on the inheritance of pathogenicity in hybrids between races of flax rust (*5*, *6*) have shown definite linkage between factors for virulence on certain varieties. All F_2 cultures of hybrids between races 22 and 24 that attacked Kenya (L^4L^4) also attacked Pale Blue Crimped (L^3L^3). Since Kenya is highly resistant to some races to which Pale Blue Crimped is moderately susceptible, the single pairs of rust-conditioning factors in these varieties are probably distinct. The factors for reaction to rust in Newland and Billings are allelomorphic, and factors for virulence on these varieties are linked. Virulence on Akmolinsk (N^1N^1), Abyssinian (N^2N^2), and Leona (N^3N^3) also was inherited as a unit in the hybrids between races 22 and 24. The single pair of rust-conditioning factors in each of these varieties appears to be distinct, as Akmolinsk is susceptible to several races to which Abyssinian and Leona are resistant and Leona is resistant to some races that attack both Akmolinsk and Abyssinian. The distinctive character of the factors for rust reaction is further emphasized by pathogenicity tests of F_2 cultures of race 6 × race 22 (hybrid A) (*6*). In this cross both parental gametes possessed a factor for virulence on Akmolinsk, but only the race 22 gamete possessed factors for virulence on Abyssinian and Leona. Each of the 74 F_2 cultures studied was virulent on Akmolinsk, but only 17 on Abyssinian and Leona. In the case of Kenya and Pale Blue Crimped, Newland and Billings, and Akmolinsk, Abyssinian, and Leona there was a parallelism of linkage between factors for virulence in the pathogen and those for rust reaction in the host. However, the linkage between factors for pathogenicity in the fungus extended beyond the linkage groups in the host. Thus, in a hybrid of a race 6 gamete (avirulent on Buda, virulent on Akmolinsk) with a race 24 gamete (virulent on Buda, avirulent on Akmolinsk) none of the 96 cultures studied was virulent on both Buda and Akmolinsk (*5*). In hybrids between race 22, to which Buda was highly susceptible, and races 6 and 24, to which Buda was resistant and moderately susceptible, respectively, all cultures to which Buda was highly susceptible had the race 22 factors for virulence on Akmolinsk, Abyssinian, and Leona (*6*). The Buda factors for rust reaction are in the *LL* and *MM* allelomorphic series and those of Akmolinsk, Abyssinian, and Leona in the *NN* linkage group.

A knowledge of the intraaction and interaction of factors for rust reaction in the host and those for pathogenicity in the pathogen should assist in programs to develop rust-resistant varieties of crop plants by indicating the potential pathogenicity of new races and by serving as a guide in the selection of parental material by indicating the possibility of incorporating specific rust-conditioning factors into the progeny.

The studies on the genetics of pathogenicity in *Melampsora lini* (*6*) give little indication that there are inherent limitations to the pathogenic capacity of races developed through hybridization of existing races. Virulence on all varieties except those possessing the Wil-

liston Brown M^1M^1 factor was inherited as a recessive character. Race 22 attacks all varieties with which it has been tested except those having the J. W. S. L^2L^2 and the Bombay *NN* factors. Consequently, it already possesses the homozygous recessives for virulence on all of the varieties having known linkage relations which might impede the accumulation of factors for virulence. The factors of race 24 for virulence on Bombay are inherited independently of all factors for virulence possessed by race 22 including those on Akmolinsk, Abyssinian, Leona, and Tammes Palé Blue, varieties having rust-conditioning factors in the Bombay *NN* linkage group. The inheritance of factors for virulence on J. W. S. has not been determined, but Vallega (*11*) reported the isolation in Argentina of a race of *M. lini* attacking J. W. S. in addition to the varieties attacked by race 22. Thus, resistance to all known races and to those that have been proved to be theoretically possible can be obtained only by combining the J. W. S. and Bombay factors for rust reaction. The possibility of combining in one race factors for virulence on J. W. S. and Bombay has not been explored, but the studies thus far completed indicate no impediment to the occurrence of such a race.

At least 19 distinct factors condition reaction of the 16 rust-differentiating varieties of flax. All but 8 of these condition immunity from or resistance to the races of *Melampsora lini* that have been isolated from North American rust collections. Consequently, there is an abundance of parental material resistant to North American races. The problem would become complicated if races having the pathogenic capacities of those obtained from South America should become established. Races attacking Bombay, the only variety known to be immune from all South American races, occur in North America. In the flax-growing region of the Midwestern States, sexual reproduction and probably hybridization precede the initiation of rust infection each year. This would afford an opportunity for the development of races having almost infinitely varied pathogenic capacities.

The fact that sexual reproduction precedes infection in the midwestern flax-growing region may have some ameliorating consequences. Virulence is predominantly recessive so that hybrid races combine the avirulence of their parents. This may account for the predominance in the Midwest of races having a restricted varietal host range (*3*). All collections obtained from Oregon, Argentina, Brazil, and Uruguay have carried races attacking several or most of the differential varieties. In these regions rust may persist throughout the year in the uredinial stage, permitting the perpetuation of the more vigorous races having the widest host range.

At the present time, any one of 15 or more factors satisfactorily conditions resistance to North American races. However, because of the possibility of the development or introduction of new races the feasibility of incorporating multiple resistance factors into new varieties should be considered. By proper selection of parents from varieties listed in table 7, it should be possible to obtain lines having 3 or 4 pairs of rust-conditioning factors, 1 each from the *LL*, *MM*, and *NN* series and 1 of the unplaced factors from Bolley Golden or Morye. From the data presented in this paper it appears unlikely that more than 1 pair of the *LL* or *MM* factors can be incorporated into a line. Since evidence of crossing over occurred in the *NN* series, it may be possible to obtain a doubling up of the factors in this series.

Although the occurrence of rust-conditioning factors in allelomorphic series limits the number of factors that may be incorporated into a variety, a knowledge of the interaction of these factors may facilitate a breeding program. Since resistance to rust invariably has been inherited as a dominant character, all the progeny of crosses of varieties having allelomorphic factors for resistance to races with which the investigator must contend should be resistant to these races. In crosses between parents having allelomorphic factors, all hybrids susceptible to a race attacking one parent but not the other should be homozygous for factors conditioning resistance to races having the reverse reaction. For example, the progeny of Newland (MM) × Bolley Golden (M^3M^3), both of which are immune from all races found in the Midwest, should be immune in that region. In this same cross all F_2 plants susceptible to race 58, to which Bolley Golden is susceptible and Newland immune, should be homozygous for the Bolley Golden M^3M^3 factors. Conversely, all F_2 plants susceptible to race 47, to which Newland is susceptible and Bolley Golden immune, should be homozygous for the Newland MM factors. Thus, it is possible, by testing the reaction of F_2 plants of hybrids having allelomorphic factors with appropriate races, to eliminate all plants not homozygous for the desired rust-conditioning factors. This permits discarding approximately three-fourths of the material before it reaches the F_3 generation.

SUMMARY

The interaction of factors conditioning reaction to rust in 20 flax varieties, including the 16 that have served as differentials for the identification of physiologic races of flax rust (*Melampsora lini*), was studied by determining the reaction of F_2 populations.

By the use of selected races of the pathogen it was demonstrated that at least 19 pairs of factors are involved in conditioning the reaction to rust of the 16 differential varieties. Of the 19 pairs of factors 16 lie in 3 series, or linkage groups: 7 in the Ottawa 770B, or LL, series; 4 in the Newland, or MM, series; and 5 in the Bombay, or NN, series. There was no indication of crossing over between factors in the LL or MM series, and factors in each of these series are considered to be allelomorphic. Some crossing over and irregular segregation ratios were obtained in hybrids between varieties having rust-conditioning factors in the NN series, and these are considered to be linked rather than allelomorphic. Additional rust-conditioning factors were found in the 4 other varieties studied.

Varieties in which single pairs of factors in the LL series condition reaction to rust are Ottawa 770B, J. W. S., Pale Blue Crimped, and Kenya. One of the two pairs of rust-conditioning factors in Buda, Williston Golden, and C. I. 438 lies in the LL series as does one of the three pairs of factors in Morye.

Single pairs of factors in the MM series condition reaction to rust in Newland, Williston Brown, and Billings. One of the two rust-conditioning factors in Buda, Williston Golden, Bolley Golden, Italia Roma, and C. I. 416–3 lies in the MM allelomorphic series. Probably the MM factors in Williston Brown and Williston Golden are identical, and those in Bolley Golden and Italia Roma may also be.

Single pairs of factors in the NN linkage group condition reaction to rust in Bombay, Akmolinsk, Abyssinian, Leona, and Tammes Pale

Blue. Bombay and Punjab appear to have identical factors. One of the two pairs of rust-conditioning factors in Italia Roma may lie in the *NN* linkage group.

One pair of rust-conditioning factors in Bolley Golden and two pairs in Morye have not been placed. Hybrids between the latter and varieties with factors in the *NN* group were not studied.

Three pairs of factors condition reaction to rust in Rio, one pair apparently lying in each of the allelomorphic series or linkage groups.

There is a parallelism in linkage between certain factors for rust reaction in the host and those for pathogenicity in the fungus. Virulence on Pale Blue Crimped (L^3L^3) and Kenya (L^4L^4), varieties having allelomorphic factors, was inherited as a unit, as was virulence on Akmolinsk (N^1N^1), Abyssinian (N^2N^2), and Leona (N^3N^3). However, pathogenicity to these three varieties having factors in the *NN* series was also linked with that to Buda ($L^1L^1M^2M^2$), indicating that linkage between pathogenic factors transcends the linkage groups conditioning resistance in the host.

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EMBRYOLOGY OF PECAN [1]

By J. W. McKay

Associate cytologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

Part of the fruits of a pecan tree frequently drop at some stage of development between pollination and maturity. The formation of the embryo, usually referred to as filling, also is sometimes faulty and incomplete. Several studies have been made on the development of the nut of the pecan (*Carya illinoensis* (Wang.) K. Koch), but investigators have not agreed on the identity and rate of development of certain embryological structures. In order to study effectively these problems it is necessary to identify the embryological tissues in the nut and to know the rate and method of their development. Without such information it is difficult, for example, to determine the physiological processes involved in the transfer of elaborated food into the kernel.

The purpose of the present investigation was (1) to trace the morphological changes that take place in the pecan nut from the time of pollination to maturity and (2) to clarify the relations between the endosperm and the embryo. This paper deals primarily with the later stages of nut development during which the embryo is formed.

Woodroof and Woodroof (*13*) [2] and Woodroof (*15*) described the early embryology of the pecan, but these writers did not agree with Shuhart (*10*) as to the time of fertilization or the beginning of embryo growth. Langdon (*6*) described the development of the ovule and fruit structures of *Carya glabra* (Mill.) Sweet, a related species of hickory. The embryology of *Juglans regia* L., another important species of the Juglandaceae, reported by Nast (*7, 8*), is similar in many details to that of pecan although the chronological order of development is quite different. Finch and Van Horn (*5*) gave a complete account of the later stages of nut filling in the pecan and discussed the physiological conditions of the tree that are favorable to the production of well-filled nuts.

MATERIALS AND METHODS

Pistillate flowers and fruits of the Greenriver variety of pecan were collected at Beltsville, Md., at weekly intervals from May to November. The Greenriver pecan is one of the northern varieties that originated in southern Indiana. Because of its origin the total develop-

[1] Received for publication December 14, 1945.

[2] Italic numbers in parentheses refer to Literature Cited, p. 283.

mental period of its nuts is about a month less than that of nuts of southern varieties; during average seasons this variety produces fairly well-filled nuts in Maryland. In addition, young fruits of the Schley variety from the time of blossoming to 49 days after pollination were available from Albany, Ga., for study.[3]

After the extraneous ovarian tissue had been removed, ovules in early stages of development were fixed in Langlet's modification of Navashin's solution. Large fruits were fixed in formalin-aceto-alcohol; they were more easily dissected after fixation than when fresh, and fixation of tissue was satisfactory for studying gross changes in the endosperm and embryo. Tissue was dehydrated in the ethyl-butyl-alcohol series, embedded in paraffin, cut 10μ to 15μ thick, and stained in iron-alum haematoxylin or safranine and fast green.

Photomicrographs of young ovule sections were made with a Zeiss Miflex camera, 9 × ocular and 8- and 4-mm. objectives. Sections of the large ovules were photographed with a Bausch & Lomb H camera with Micro Tessar lenses. Mature fruits were sectioned by hand with a sharp knife and photographed on a ground-glass surface with illumination from below to avoid shadows. Some of the dissected ovules were stained lightly in aqueous safranine and photographed in water, as the delicate endosperm at certain stages is formless if removed from a supporting liquid.

Some fruits on a particular tree were as much as a week or 10 days later in endosperm development than others on the same tree; it was therefore necessary to use the average of a number of fruits collected on any one date as representative of that weekly stage of development. The same procedure was also necessary in regard to weekly stages of embryo growth.

POLLINATION

Pollination occurs about June 1 in Maryland, the exact time depending upon the season and variety. At the time of pollination a fully formed embryo sac is present in the nucellus of the pecan ovule (fig. 1, *A*). Pistillate flowers of the variety Schley grown at Albany, Ga., also contain mature embryo sacs at the time of stigma receptivity. The method of embryo-sac formation is similar to that described by Woodroof (*15*) except that all of the stages appear to be completed prior to stigma receptivity. Four megaspores are formed by the megaspore mother cell about 10 days before pollination, and during this 10-day period the chalazal megaspore through three successive nuclear divisions forms the normal angiosperm type of embryo sac as described by Sharp (*9*). Woodroof (*15*) found that four megaspores were present in the nucellus at the time of stigma receptivity and the embryo sac matured a week later. Shuhart (*10*) reported an eight-nucleate megagametophyte in pecan at the time of pollination; fusion of the two polar nuclei occurred immediately after the eight nuclei of the embryo sac were fully differentiated.

The course of pollen tubes through the tissue of the stigma, style, and ovary was difficult to follow with the fixatives and stains used. It was not possible to determine whether pollen tubes normally enter

[3] Acknowledgement is due Max B. Hardy, U. S. Department of Agriculture Pecan Laboratory, Albany, Ga., for supplying this material.

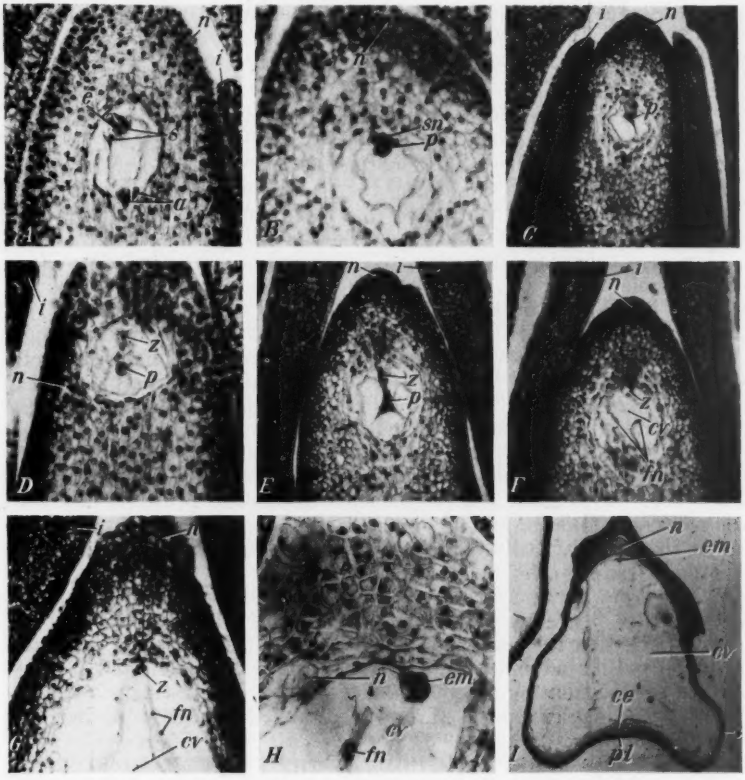

FIGURE 1.—Longitudinal sections of ovules of Greenriver and Schley pecans, showing various stages of development. *A*, Mature embryo sac at time of pollination, June 1. *B*, Embryo sac 4 days after pollination, showing primary endosperm nucleus and union of egg and sperm, or syngamy. *C*, Primary endosperm nucleus and slightly out-of-focus zygote 4 days after pollination. *D*, Primary endosperm nucleus and zygote 6 days after pollination. *E*, Primary endosperm nucleus and zygote with single integument enclosing the nucellus 10 days after pollination. *F*, Dormant zygote with endosperm beginning to develop. *G*, Endosperm more extensively developed than in *F* and dormant zygote 42 days after pollination. Collapse of the central vacuole due to fixation caused shrinkage of the endosperm from a peripheral position in the embryo-sac cavity. *H*, Embryo of approximately eight cells 48 days after pollination, July 19. *I*, Small, undifferentiated embryo and greatly expanded endosperm 62 days after pollination, August 2. The central vacuole occupied most of the ovule, and cellular endosperm appeared adjacent to the seed coat. The nucellus was reduced to a small cap of tissue at the micropylar end of the ovule. *A*, *D*, *H*, × 174; *B*, × 186; *C*, *E*, *F*, *G*, × 90; *I*, × 10. *a*, Antipodals; *ce*, cellular endosperm; *cv*, central vacuole of the endosperm; *e*, egg; *em*, embryo; *fn*, free nuclei of the endosperm; *i*, integument; *n*, nucellus; *p*, primary endosperm nucleus; *pl*, placenta; *s*, synergids; *sn*, syngamy; *z*, zygote.

the ovule through the micropyle or the chalaza, although they were usually recognizable after they entered the cavity of the embryo sac.

The ovule is orthotropous and sessile and has a single integument that encloses the nucellus in 4 to 10 days after pollination (fig. 1, *C*

and *E*). The integument later becomes the thin, brown, membranous seed coat, or outer covering of the kernel.[4]

FERTILIZATION

Union of the polar-fusion nucleus with one of the gametes from the pollen tube takes place about 4 days after pollination (fig. 1, *C*). The large primary endosperm nucleus thus formed is the most prominent feature of the embryo sac during the 14-day period after pollination (fig. 1, *B–E*). It lies embedded in a thick strand of cytoplasm near the center of the sac, with large vacuoles on either side.

Union of the second male gamete and the egg occurs 4 to 7 days after pollination (fig. 1, *B*). Long-delayed fertilization of the egg as reported by Woodroof and Woodroof (*13*) was not observed. The interval between fertilization of the egg and that of the polar-fusion nucleus by the two male gametes may be a matter of hours, as both fusions were observed in ovules fixed 4 days after pollination (fig. 1, *B*, *C*). Langdon (*6*) stated that in *Carya glabra* the polar-fusion nucleus united with the male gamete only a few hours before fusion of the egg and the second male gamete. Woodroof (*15*) reported union of the polar-fusion nucleus and the male gamete in *C. illinoensis* 14 days after pollination and fertilization of the egg probably 35 to 42 days after pollination. The present studies show that for 7 to 14 days after the egg is fertilized the synergids remain as dark-staining, irregularly shaped cells.

The zygote remains inactive at the micropylar end of the embryo sac about 42 days after pollination (fig. 1, *G*). The length of this inactive period varies somewhat, depending upon the time the pistillate flower is pollinated and other factors, as some ovules collected 49 days after hand-pollination showed the zygote still in a resting stage. In none of the ovules examined, however, was the inactive period less than 42 days.

GROWTH PERIODS OF THE FRUIT

The development of the pecan fruit subsequent to the first 14 days after pollination may be divided into two approximately equal periods: (1) Endosperm development, which requires about 77 days; and (2) embryo growth, or filling, which requires about 70 days. This division of the total growth period on the basis of morphological features corresponds almost exactly to the division made by Crane and Hardy (*3*) on the basis of growth behavior. These writers reported two periods in the development of the pecan fruit: (1) The period of growth in size, which begins at blossoming and probably ends with hardening of the shell, at which time the nuts have attained full size; and (2), the period of filling of the nut, which probably begins about the time the shell begins to harden and ends with separation of the nut from the shuck. In the present investigation it was found that the stage of maximum endosperm development occurs just as the shell begins to harden at the blossom end of the fruit and the embryo reaches macroscopic size. Thus, the beginning of shell hardening

[4] Since the term "kernel" is widely used to indicate the edible portion of the pecan nut, it should be understood that it includes at maturity the seed coat, embryo, and remains of the endosperm.

coincides with maximum endosperm development and the beginning of rapid embryo growth, or filling.

The different growth periods of fruit and seed are the most striking features of pecan-fruit development. The fruit enlarges during the first 56 days after pollination to about half its full size before there is macroscopic evidence of ovule enlargement. During the third month after pollination ovule enlargement is accelerated, and at the end of this period both ovule and fruit are approximately full-sized. Filling of the nut, which means rapid enlargement of the embryo, begins about the time the fruit and ovule attain maximum size.

ENDOSPERM DEVELOPMENT

The primary endosperm nucleus starts dividing about 14 days after pollination, or about the middle of June in Maryland (fig. 1, *F*). A rapid increase is noted in the size of the embryo-sac cavity and growth of the endosperm 28 days after initiation of endosperm growth, 42 days after pollination (fig. 1, *G*). From the beginning the most striking feature of the endosperm is the large central vacuole filled with liquid. During the early stages of ovule growth the free nuclei of the endosperm are embedded in a strand of cytoplasm along the outer margin of the central vacuole and in contact with the nucellus. The layer of endosperm tissue in figure 1, *G*, was withdrawn from the margin of the cavity by the fixative.

Growth of the endosperm is free-nucleate around the outer margin of the central vacuole for about 42 days, or until approximately August 1, about 60 days after pollination. At this time cell walls are formed beginning at the chalazal end of the ovule just above the placenta and progressing along the outer margin of the central vacuole to the micropylar end. The ovule is 5 to 7 mm. long at this time (fig. 2, *A*, *i*). A longitudinal section of an ovule collected on August 2 shows a thin layer of cellular endosperm around the outer margin of the central vacuole and adjacent to the seed coat (fig. 1, *I*). At this stage the nucellus except for a small cap of tissue at the micropylar end has been absorbed by the rapidly expanding endosperm.

The progressive development of the ovule, particularly the endosperm and the embryo, throughout the season is shown in chronological order in figures 2 and 3. Enlargement of the ovule is very rapid during the latter part of July and early August (fig. 2, *A*, *h–k*). This is also the period of most rapid increase in fruit size. Growth of the ovule during this period is accompanied by a striking enlargement of the central vacuole, and there is also some increase of cellular endosperm tissue on the inner side of the seed coat. Under normal conditions the ovule is very turgid during this period because of its watery contents; the pressure developed by the central vacuole is probably the force that pushes the elongating ovule into the lower part of the nut, as suggested by Woodroof and Woodroof (*13*). This force is so great that it is practically impossible to dissect fresh fruits during this period without breaking the seed coat and endosperm tissue and allowing the vacuole contents to escape and the ovule to collapse. Fruits preserved in formalin-aceto-alcohol are much easier to dissect, as the fixative destroys the turgidity of the central vacuole.

At the time shell hardening begins, about the last week in August, the fruit is almost full-sized (fig. 2, *B*, *d*) and the central watery vacu-

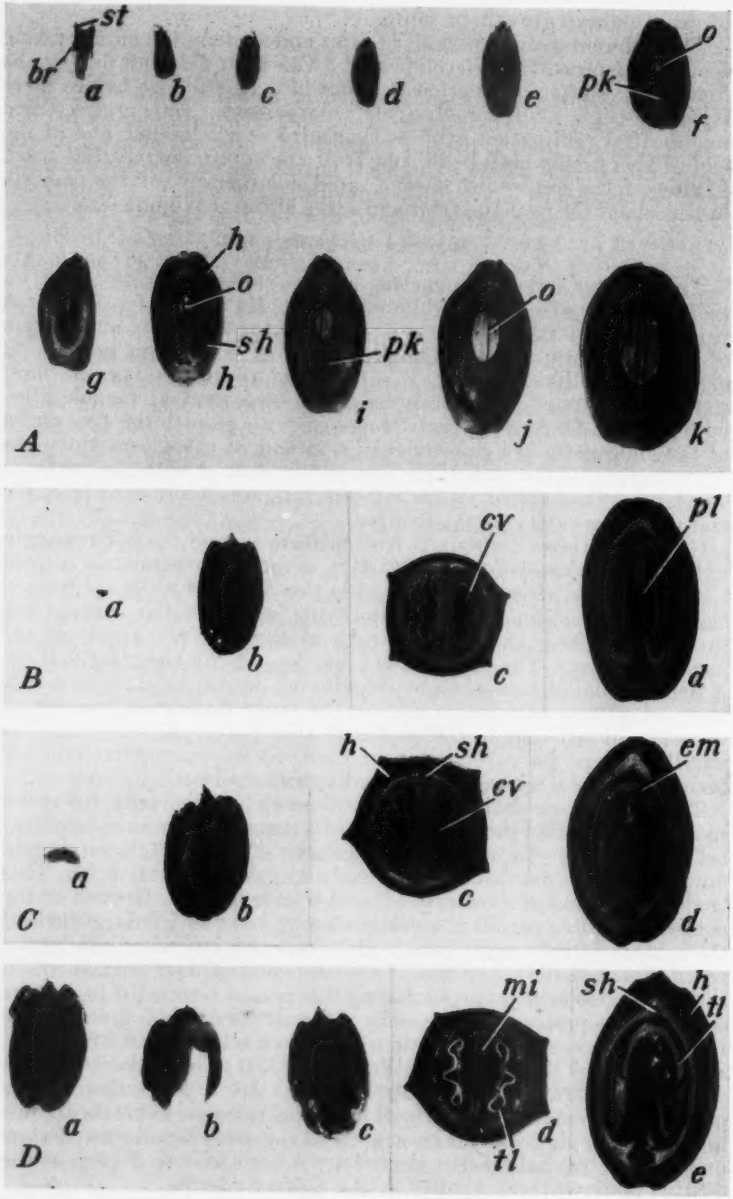

FIGURE 2.—For explanatory legend see opposite page.

ole of the endosperm has reached its greatest proportion. At this stage the ovule is more than two-thirds of its full size and is very turgid because of its liquid contents. Thor and Smith (*12, p. 109*) referred to this stage as "the kernel in the watery stage." The embryo, which first becomes macroscopic at this time, may be observed as a small bit of solid white tissue about 1 to 2 mm. long in the micropylar tip of the ovule (fig. 2, *B*, *a*, *d*). Since the embryo enlarges rapidly from this point on and the endosperm has reached its maximum development, the beginning of shell hardening is a convenient marker to separate approximately the two main phases of seed formation, namely, endosperm development and embryo growth, or filling. The period of growth in size of the nut as reported by Crane and Hardy (*3*) is then correlated with endosperm development, and their period of filling of the nut is correlated with embryo growth.

Beginning about the first week in September, as the ovule attains full size, a gelatinous layer of cellular endosperm is formed on the inner wall of the seed coat. However, in fruits collected on September 2 this membranous layer was still too fragile to be dissected from the ovule (fig. 2, *C*, *c*, *d*). In fruits collected on September 8 the peripheral cellular portion of the endosperm was sufficiently well developed to permit dissection from the ovule in fixed material. In figure 2, *D*, *c*, is shown the seed coat of an ovule from which the endosperm (*a*) and embryo (*b*) were dissected. The central vacuole of the endosperm is greatly reduced at this stage, as may be observed by comparing figure 2, *D*, *d*, with *C*, *c*. This reduction is due to encroachment of the embryo and a rapid increase in the cellular portion of the endosperm. The cellular endosperm at this stage is transparent and very homogeneous in structure. In order to photograph the tissue it was necessary to stain it lightly in 1-percent aqueous safranine and to submerge it in water so that it would retain its shape. In figure 2 it may be noted that the cellular endosperm (*D*, *a*) conforms to the shape of the ovule (*D*, *c*), indicating the peripheral position of the former within the seed coat. The endosperm apparently at this stage of development was described by Woodroof and Woodroof (*13, p. 1057*) as a "thick succulent pad of material" included within the folds of the cotyledons, the origin and purpose of which "has not been determined."

From this time on the endosperm is rapidly surrounded by the folds of the cotyledons and within 14 days—by September 22—was absorbed and reduced to a thin membrane (fig. 3, *C*, *a*; *D*, *a*; *E*, *a*; and fig. 4, *B*).

FIGURE 2.—Pecan-fruit structures showing chronological order of development; × approximately 1. *A*, Longitudinal sections of fruits in plane of the middle septum showing approximately weekly stages of growth and increase in size of fruit and ovule, beginning 6 days after pollination (*a*) and ending 77 days after pollination, August 17 (*k*). *B*, Dissected fruits collected August 25: *a*, Embryo; *b*, seed coat; *c*, cross section showing central vacuole of the ovule; *d*, longitudinal section perpendicular to middle septum showing placenta and ovule two-thirds its full length. *C*, Dissected fruits collected September 2: *a–d*, Same as in *B*. *D*, Dissected fruits collected September 8: *a*, Endosperm dissected from within folds of the cotyledons and slightly stained in 1-percent aqueous safranine; *b*, embryo with cotyledons slightly more than half as long as the ovule; *c*, seed coat; *d*, cross section showing white solid tissue of the cotyledons and reduced endosperm; *e*, longitudinal section showing cotyledons extending about half the length of the ovule. *br*, Bract; *cv*, central vacuole of endosperm; *em*, embyro; *h*, hull; *mi*, middle septum; *o*, ovule; *pk*, packing tissue of ovary; *pl*, placenta; *sh*, shell; *st*, stigma; *tl*, cotyledon lobe.

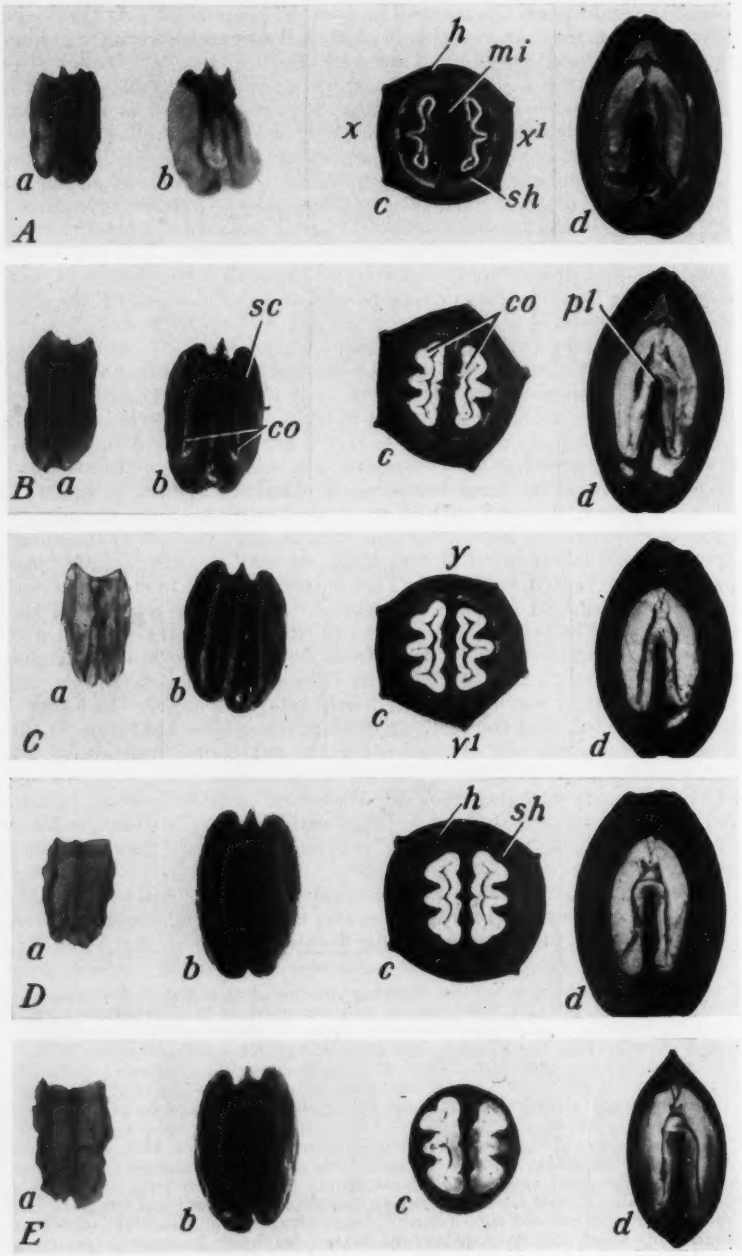

FIGURE 3.—For explanatory legend see opposite page.

Woodroof and Woodroof (*13, p. 1057*) stated that "Included within the folds of each cotyledon is a thin white membrane containing numerous vascular bundles." In the present work no vascular bundles were observed in the endosperm at any stage of its development. The tissues become crushed within the folds of the cotyledons in the later stages of filling, and the folds and creases thus developed superficially resemble vascular bundles (fig. 3, *D*, *a*, and *E*, *a*). However, close inspection of stained histological sections revealed no trace of vascular tissue and showed the endosperm to be a homogeneous and undifferentiated tissue (fig. 4, *A* and *B*).

EMBRYO GROWTH, OR FILLING

Early Stages

The first division of the zygote occurs about 42 days after pollination, or near the middle of July in Maryland. Two- to eight-celled embryos were observed in ovules fixed July 19 (fig. 1, *H*). Woodroof (*15*), working in Georgia, found that the first division of the zygote occurs about 56 days after pollination, and in an earlier work Woodroof and Woodroof (*13*) observed two- to four-celled embryos 70 days after pollination. Shuhart (*10*) did not record the exact time interval between pollination and zygote division but stated that the embryo begins to develop about September 1 at Stillwater, Okla. The details of tissue differentiation in very young embryos were not studied in the present work, emphasis being placed on the mechanism of the filling of the kernel in the later stages of embryo growth.

The embryo was a round mass of undifferentiated tissue in ovules collected on August 2, approximately 21 days after the first division of the zygote (fig. 1, *I*). A suspensor is formed in very young embryos but consists of only a few small cells that are soon crushed by the growing embryo (fig. 5, *C*). During late July and early August the basal portion of the ovule rapidly pushes downward into the ovarian cavity on either side of the middle septum. The fruit reaches approximately half its growth in size by the first of August (fig. 2, *A*, *i*).

The differentiation of cotyledons is evident in the embryos of fruits collected August 18; the meristems of the epicotyl and root axis are also recognizable (fig. 5, *C*). The embryo is still microscopic, however, and the much enlarged ovule is filled with the watery endosperm. By August 25, the embryo is large enough to be seen with the unaided eye (fig. 2, *B*, *a*, *d*). Growth in size of the ovule and fruit is almost complete at this time, and the tip of the shell at the blossom end of the fruit has begun to harden. For convenient reference the length of the

FIGURE 3.—Pecan-fruit parts showing endosperm absorption and embryo growth after the middle of September; × approximately 1. *A*, Dissected fruits collected September 15: *a*, Endosperm removed from within folds of the cotyledons and slightly stained in 1-percent safranine; *b*, kernel with seed coat mostly removed; *c*, cross section showing cotyledons somewhat thicker and endosperm more reduced than in figure 2, *D*, *d*; *d*, longitudinal section showing cotyledons extending almost to the basal end of the ovule. *B–D*, Dissected fruit collected approximately 1, 2, and 3 weeks later than those in *A*, showing further reduction of endosperm: *a–d*, Same as in *A*. *E*, Dissected mature nuts harvested November 7: *a*, Thin, membranous endosperm; *b*, kernel; *c*, cross section; *d*, longitudinal section. The stages between *D* and *E* were omitted because little change occurred in the kernels during this period. *co*, Cotyledon halves; *h*, hull; *mi*. middle septum; *pl*. placenta; *sc*. seed coat; *sh*. shell; x–x^1, plane of the cotyledons (perpendicular to the middle septum); y–y^1, plane of the middle septum.

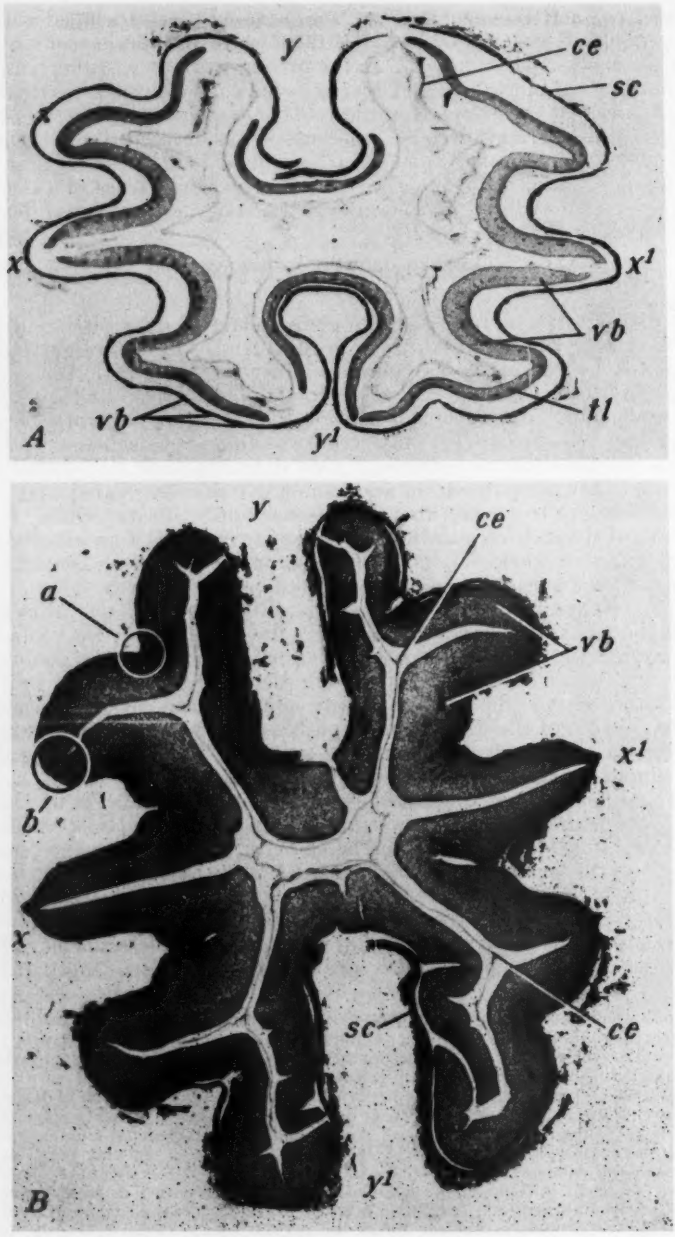

FIGURE 4.—For explanatory legend see opposite page.

embryo and other fruit and ovule structures at different dates during the season is given in table 1.

TABLE 1.—*Length of fruit and seed structures of the pecan, variety Greenriver, at certain dates during the growing season of 1941, Beltsville, Md.*

[Length given in millimeters]

Date	Period after pollination	Fruit	Ovule	Endosperm	Embryo
	Days				
June 1	0	8	0.5		
June 14	13	12	1	2–4 nuclei	Zygote.
July 12	41	20	4	2	Do.
July 19	48	25	5	3 [1]	2- to 8-celled.
July 26	55	30	6	5	Many-celled.
Aug. 9	69	40	15	14	0.5.
Aug. 25	85	40	25	25 (water stage)	2.
Sept. 8	99	40	30	Succulent-pad stage	15.
Sept. 22	113	40	30	Much reduced	30.
Nov. 7	[2] 159	40	30	Thin membrane	30.

[1] Cellular endosperm appeared.
[2] Mature nut.

Growth in Length of Cotyledons

In Maryland the month of September is the period during which the most rapid embryo growth occurs. Fruits collected September 2 contained embryos 4 to 6 mm. long with bilobed cotyledons oriented perpendicular to the plane of the middle septum (fig. 2, *C*, *a*). The cotyledons elongate at first by means of a terminal meristem at the lower margin of the lobes (fig. 6, *B*). Meristematic activity is soon discontinued at the lower midpoint of each cotyledon, which forms an apical notch similar to that in the embryo of *Juglans regia* described by Nast (*8*). The apical meristem of the cotyledons is present in only very young embryos, and when the cotyledons are 2 to 4 mm. long meristematic activity is present throughout the tissue. From this point onward intercalary growth is responsible for rapid elongation of the cotyledons.

During the first half of September, growth of the cotyledons is largely in length rather than in thickness. The cotyledon halves follow the contour of the ovule in their downward growth and lie adjacent to the seed coat, thus enclosing and surrounding the endosperm. Each cotyledon is bisected by the middle septum, and the cotyledon halves grow into each lobe of the bilobed ovule. The four upper chambers of the ovule are filled by lateral folds of the cotyledon halves, which grow upward into these chambers as the main lobes of the cotyledons grow downward in the ovule.

FIGURE 4.—Median cross sections of pecan ovules (kernels); × 7. *A*, 99 days after pollination, September 8. At this stage the cotyledons were elongating rapidly but had not yet reached the basal end of the ovule (see fig. 2, *D*, *e*). The cotyledon lobes were adjacent to the seed coat and enclosed the endosperm. *B*, 113 days after pollination, September 22. Thickening of the cotyledons was well advanced at this stage, and the endosperm was reduced to a small area within the cotyledon lobes. New tissue was being formed largely in subepidermal meristems (deeply stained) along the outer margins of the cotyledons adjacent to the seed coat. (See in fig. 6, *A* and *C*, respectively, enlargements of the encircled areas marked *a* and *b*.) *ce*, Cellular endosperm; *sc*, seed coat; *tl*, cotyledon lobe; *vb*, vascular bundles; x–x^1, plane of hte cotyledons; y–y^1, plane of the middle septum.

Finch and Van Horn (*5, p. 440*) ascribed the early stages of filling to "the conversion of the gel into a layer of white solid material." Later a second "gel layer" is formed and this "in turn is converted into solid material which adds to and thickens the original solid layer"

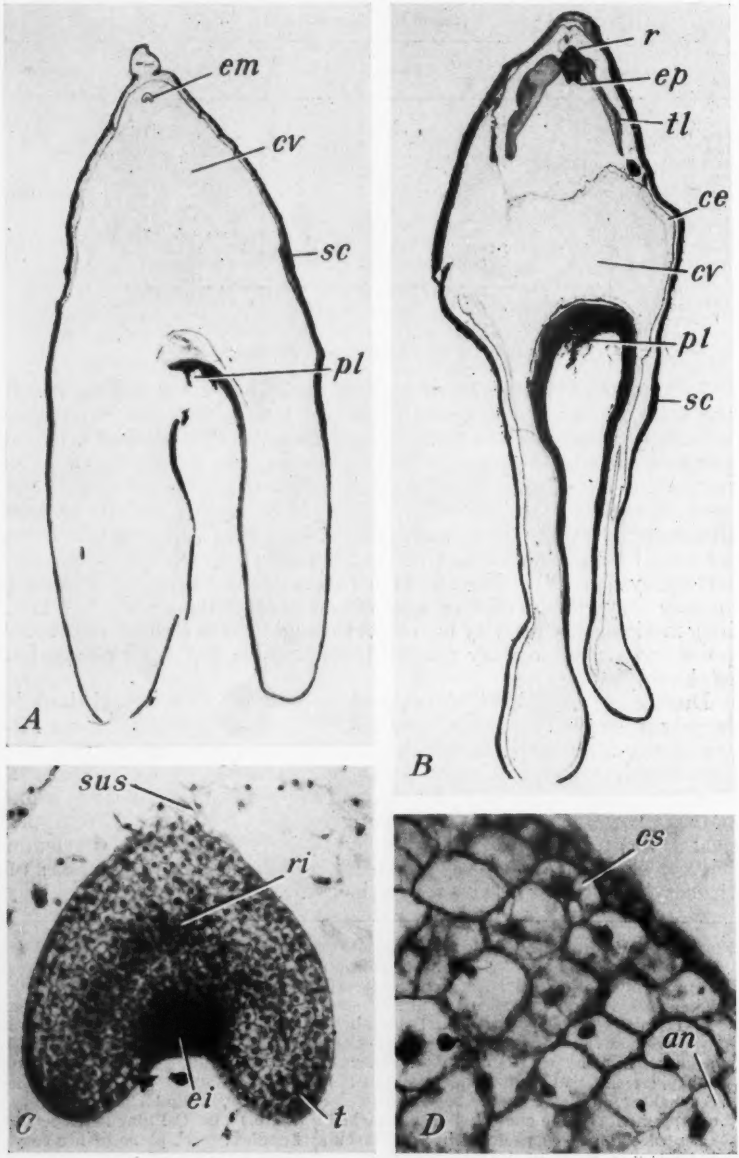

FIGURE 5.—For explanatory legend see opposite page.

(*5, p. 442*). It is easy to understand this account of the method of filling because of the rapidity with which the cotyledons extend into the lower part of the ovule. It will be noted in figure 2, *C* and *D*, and figure 3, *A*, that the cotyledons expand almost the full length of the ovule in 14 days. Furthermore, the cotyledons occupy a position adjacent to the seed coat and push the endosperm inward, thus giving rise to Finch and Van Horn's centripetal-layer explanation. It should be emphasized, however, that enlargement of the cotyledons is strictly an autonomous process and that increase in storage tissue is due to cell multiplication within rather than to a conversion process as proposed by Finch and Van Horn.

The cotyledons are relatively thin during the 14-day period of rapid elongation (fig. 2, *D*, *d;* fig. 3, *A*, *c;* and fig. 4, *A*). Cross walls of dividing cells are mostly perpendicular to the long axis of the cotyledon, resulting in elongation of the structure rather than thickening (fig. 6, *B*). The cells are highly vacuolate and have small nuclei typical of storage parenchyma (fig. 6, *D*). Nevertheless, cell division is fairly rapid and the mitotic figure is similar to that described for vacuolate cells by Sinnott and Bloch (*11*), except that cross-wall formation is accomplished by the cell-plate method. Cytoplasmic strands are prominent in these cells during the prophase of nuclear division (fig. 5, *D*).

Growth in length of the cotyledons apparently may be arrested by unfavorable nutritional conditions during the period of elongation. Nuts of the Mahan and Schley varieties with kernels showing varying degrees of shortening are illustrated in figure 7, *A*, *b*, *d*, and *D*, *b*. Some of these kernels are well-filled and solid even though they are shortened. Others, however, show air spaces within the folds of the cotyledon halves, indicating that cotyledon thickening did not progress to completion. In figure 7, *B*, *a*, is shown a cross section of a well-filled Mahan nut with full length kernel; in *B*, *b*, a shortened but solid, somewhat imperfect kernel; and in *B*, *c*, *d*, shortened poorly filled kernels. Although experimental evidence is not at hand to prove that it is true, it seems reasonable to believe that conditions of tree growth and nutrition at the time of cotyledon elongation may influence length of kernel.

FIGURE 5.—Sections of pecan ovules (kernels) and embryo tissue. *A*, Longitudinal section of ovule fixed 78 days after pollination, August 18. The embryo is microscopic and the endosperm is composed mostly of the watery central vacuole (compare with fig. 2, *A*, *k*). *B*, Longitudinal section of ovule fixed 15 days later than *A*, September 2. Lower portion of the ovule has collapsed because of rupture of the seed coat during dissection, allowing liquid of the central vacuole to escape. Peripheral cellular endosperm is more highly developed than in *A*, but still very fragile (compare with fig. 2, *C*, *d*). *C*, Embryo shown in *A*. The cotyledon lobes and the initials of radicle and epicotyl are in early stages of differentiation. *D*, Section of outer portion of cotyledon showing beginning of subepidermal meristem, September 3. The large, thin-walled cells have small nuclei and prominent cytoplasmic strands and have divided in a plane perpendicular to the surface of the cotyledon. *A* and *B*, × 6; *C*, × 144; *D*, × 321. *an*, Cell in anaphase of division; *ce*, cellular endosperm; *cs*, cell with prominent cytoplasmic strand; *cv*, central vacuole of endosperm; *ei*, epicotyl initial; *em*, embryo; *ep*, epicotyl; *pl*, placenta; *r*, radicle; *ri*, radicle initial; *sc*, seed coat; *sus*, remains of suspensor; *t*, cotyledon initial; *tl*, cotyledon lobe.

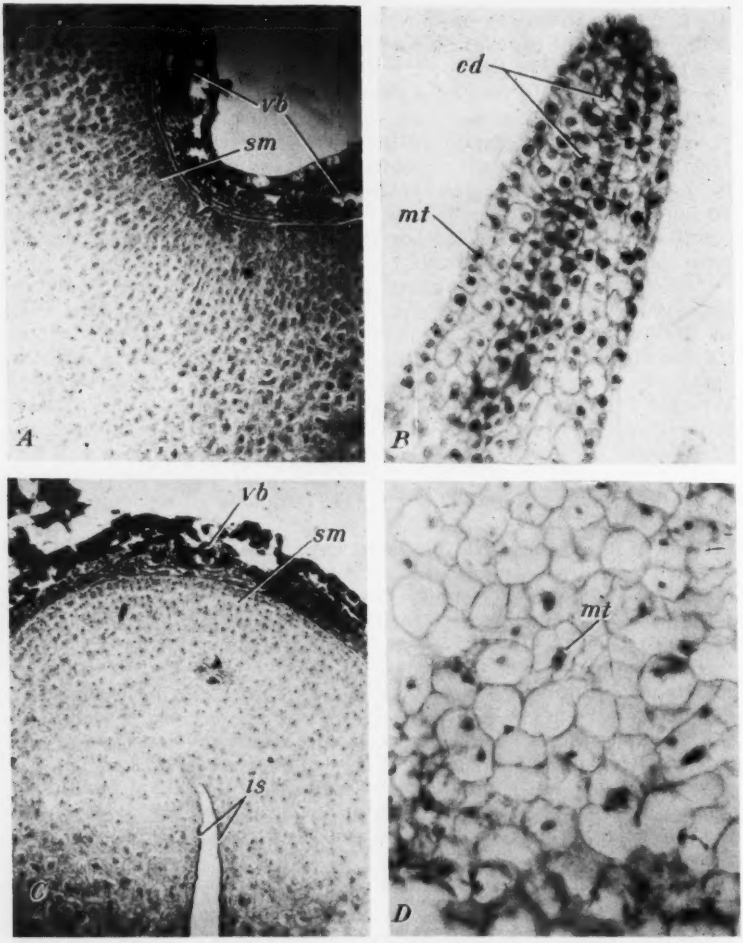

FIGURE 6.—Sections of pecan cotyledons showing meristems and cell structure. *A*, Cross section of a portion of outer surface of cotyledon opposite an inward fold of the seed coat, showing well-developed subepidermal meristem, September 22 (position of the sector is indicated by *a* in fig. 4, *B*). Tissue of the cotyledon was in contact with the seed coat from which nutrients were derived by absorption. *B*, Longitudinal section through lower edge of cotyledon in 2-mm. embryo, August 25. Rapidly dividing cells with large nuclei characteristic of meristematic tissue occurred on the lower margins (upper in photograph) of cotyledons 1 mm. long; most of the cells had divided in a plane perpendicular to the long axis of the cotyledon, resulting in elongation. *C*, Cross section through portion of cotyledon showing greater meristematic activity on the outer surface than on the inner, September 22 (position of the sector is indicated by *b* in fig. 4, *B*). *D*, Cross section showing cell division in the inner tissue of the cotyledon during the period of elongation, September 2. The cells were highly vacuolate and typical of storage parenchyma, but division was continuous throughout the tissue. *A* and *C*, × 57; *B* and *D*, × 259. *cd*, Daughter cells with cross walls perpendicular to the long axis of the cotyledon; *is*, inner surface of cotyledon; *mt*, cell in metaphase of division; *sm*, subepidermal meristem of cotyledon; *vb*, vascular bundles of seed coat.

Growth In Thickness of Cotyledons

Growth in thickness of the cotyledons is accomplished by the activity of a subepidermal meristem (fig. 5, *D;* and fig. 6, *A*, *C*), beginning at the time the cotyledons reach full length the first or second week in September. Nast (*8, p. 189*), in discussing the thickening of cotyledons in *Juglans regia*, stated:

> In fifteen-millimeter embryos a rather pronounced growth in thickness begins in the area nearest to the epicotyl and spreads to the margins. This increase in thickness is carried on by a cambium-like meristem.

She found (*pp. 189–190*) that "Storage of food begins with increase in thickness of the cotyledons and starts in the basal region."

Growth in thickness of full-length pecan cotyledons is similar to that of embryos of *Juglans regia* described by Nast (*8*). In the early stages of thickening, activity of the subepidermal meristem is greater on the inner surface of the cotyledons adjacent to the endosperm, presumably as a result of the nutritive action of the endosperm. During the later stages of filling when the endosperm is mostly absorbed, cell division is more active in the outer meristem of the cotyledons (fig. 6, *C*). At this time food materials move into the embryo along the outer margins by absorption from the seed coat. Location of meristem activity in the cotyledons thus seems to be related to location of the nutrient supply. The formation of solid kernels depends upon the complete filling of the cavity within the folds of the cotyledon halves, and in well-filled kernels meristematic activity continues in the outer and to some extent in the inner meristem until almost harvesttime.

Storage of food is greatly accelerated as the cotyledons increase in thickness. Cells in the central region of the cotyledons first show the presence of storage products, and as thickening progresses cells nearer the margin become filled with stored food. In the final stages of cotyledon thickening abundant stored food is found in the dividing cells of the meristem near the surface.

Elaborated food is translocated to the embryo by absorption from the seed coat. The embryo is probably nourished almost entirely by the endosperm until thickening of the cotyledons is under way. The seed coat has a well-developed vascular system composed of a network of bundles, which extend from the placenta to all parts of the integument (fig. 4, *A*; fig. 6, *A*, *C*). The amount of elaborated food available from the tree that moves through the vascular system of the seed coat to the embryo seems to be the factor which determines the filling of the kernel rather than the conversion of gel to solid tissue as suggested by Finch and Van Horn (*5*). Poorly filled nuts are therefore an indication that the tree is unable to supply the materials necessary for complete embryo development.

No biochemical tests were made in this work to determine the nature of storage products in the pecan seed. Finch and Van Horn (*5*) found that the gel layer, or endosperm, contained invert sugars but no fats, whereas the embryo contained abundant fats but no sugars. Thor and Smith (*12*) reported that a sharp maximum in the concentration of total sugars in the nut occurred in July and suggested that this may be related to zygote division, which, according to Woodroof and Woodroof (*13*), begins about this time. The high sugar content of the nut in Texas during July is undoubtedly related to rapid endosperm

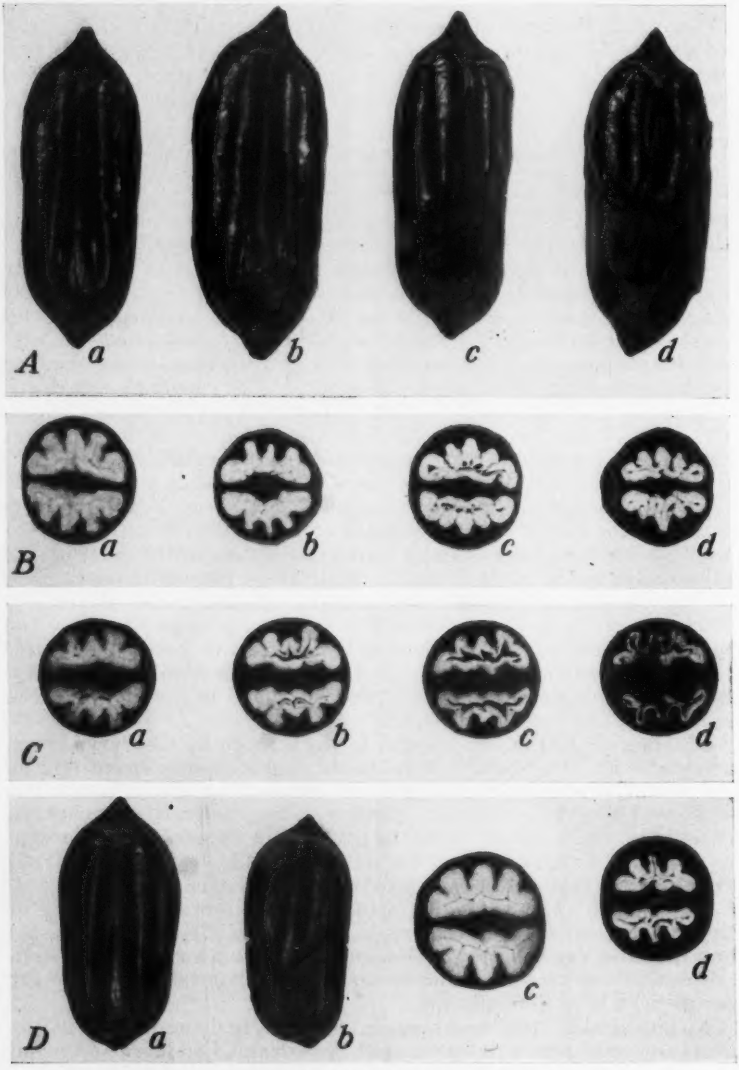

FIGURE 7.—Mature pecan nuts showing different types of filling; × approximately 1. *A*, Mahan nuts; *a*, Well-filled nut; *b-d*, nuts with shortened kernels. *B*, Cross sections of nuts shown in *A*; note in *a* and *b* that the kernels are solid and well-filled, whereas in *c* and *d* they are hollow and poorly filled. *C*, Stuart nuts: *a*, Well-filled nut; *b-d*, poorly filled nuts showing spaces resulting from lack of cotyledon thickening. *D*, Schley nuts: *a*, Well-filled nut; *b*, nut with shortened kernel; *c* and *d*, cross sections of well-filled and poorly filled nuts.

development rather than to the beginning of embryo growth as suggested by Thor and Smith (*12*). From these reports it seems clear that the endosperm is composed largely of carbohydrate material. The fact that fats appear in the embryo only after development is well under way indicates that fat synthesis takes place in the cotyledons. This is probably true of proteins as well. While it is not the purpose of this paper to discuss the physiology of nut filling, the normal course of ovule development indicates that elaborated food in soluble form is translocated to the endosperm and embryo through the elaborate vascular system of the seed coat. In the later stages of filling, the cotyledons absorb soluble food materials from the seed coat and these are transformed within the tissue of the cotyledons into fats and protein, the principal storage products of the kernel. This concept of the movement of translocated foods agrees with the statement of Thor and Smith (*12, p. 118*) that ". . . practically all of the oil content of the pecan kernel is formed from materials brought into the fruit from other parts of the tree at the time of oil formation." Woodroof, Woodroof, and Bailey (*14, p. 38*) also concluded: "Unfilled or poor quality nuts are directly related to unfavorable growing conditions the last month before harvest."

The morphological details of tissue differentiation in the hypocotyl and epicotyl were not determined in this study, because these structures are small and have no significance as storage organs.

DISCUSSION

One of the most striking features of pecan-nut development is the long period of endosperm enlargement before there is visible evidence of embryo growth. The first division of the zygote occurs about 28 days after the endosperm starts to grow and about 42 days after pollination. During this period of zygote dormancy the failure of initiation of endosperm development or of its continuation would undoubtedly result in dropping of the fruit. The large increase in drop of nuts under cellophane bags as compared with that of unbagged nuts observed by Crane et al. (*4*) during the early periods of nut development may possibly be explained as the result of endosperm failure. In this case the unfavorable environment within the bags (high temperature and humidity) probably caused the delicate endosperm to cease development and the fruits to drop. The unbagged clusters showed the greatest rate of drop during August and September at the height of endosperm development and after embryo growth was well under way. During this period the endosperm is composed of a layer of fragile cellular tissue surrounding a watery central vacuole. The thin-walled cells of the cellular endosperm are undoubtedly easily ruptured, and unfavorable environmental influences, such as hard winds and high temperature, could cause injury to a few of these cells. Oxidation of this tissue, indicated by discoloration, undoubtedly spreads rapidly after such injury and causes the nut to

drop. Crane et al. (*4*) observed that dropped nuts showed internal discolored areas which appeared to be ruptured tissues, and they concluded as follows (*p. 42*):

> The evidence indicates that the nuts are most susceptible to injury from external influences during the period from about June 1 to September 1, when they are in the watery stage, and that these external factors are most injurious when they alter the environment most rapidly.

While endosperm failure is difficult if not impossible to demonstrate experimentally it seems clear that this may be one of the large factors in nut drop, at least in the first half of the growing season.

Brink and Cooper (*1, 2*) described a type of seed failure in alfalfa caused by the inability of the endosperm to keep pace with growth in the surrounding tissues of the seed. As a result, the maternal tissue at the chalazal end of the embryo sac produces abnormal growth that causes collapse of the ovule. This type of failure, which is termed "somatoplastic sterility," occurred in 34.4 percent of self-pollinated ovules as compared with only 7.1 percent of cross-pollinated ones. The greater percentage of seed set after cross-pollination is attributed to the added impetus to growth of the endosperm resulting from cross-fertilization, or hybridity. Brink and Cooper (*1, 2*) stated that this type of seed failure is probably a widespread, although rather obscure, form of sterility in plants. Since pecan, like alfalfa, is adapted to cross-pollination, the type of endosperm failure which they described may cause some of the drop of pecan fruits early in the season. Self-pollination probably occurs rather frequently in certain varieties, especially in certain seasons when dichogamy is not very pronounced, but little information is available on this point. Unpublished results indicate that the set of nuts in certain varieties after self-pollination is much smaller than after cross-pollination, and many of the self-pollinated nuts are empty at maturity. Further study of nuts that drop after artificial self-pollination and those that drop from natural causes should be made to determine whether or not endosperm failure is responsible for the rather heavy drop of nuts that frequently occurs during the first half of the growing season.

One of the most probable causes of endosperm failure is the high degree of turgidity that persists within the ovule throughout endosperm development. The tension set up by the large watery vacuole undoubtedly causes the peripheral cellular tissue to be more susceptible to injury from environmental influences. Crane et al. (*4, p. 39*) reported that "Schley nuts sometimes split open when the developing kernel is in the 'watery stage' . . . after a period of drought followed by heavy rains." In this case the pressure developed in the endosperm as a result of water becoming suddenly available was so great as to cause the longitudinal rupture of the seed coat, shell, and shuck and the release of the liquid endosperm. Such injured nuts drop within 3 to 5 days. On the other hand, a period of continued drought at any time during the period of development is likely to affect the size or filling of the nuts or both because moisture is withdrawn and withheld from the nuts by the leaves during periods of excessive transpirations. It is of the greatest importance, therefore, that a uniform moisture supply be available to the tree during the main period of nut growth and filling in order that the nut may attain good size and the tissues of the endosperm and embryo may develop at an optimum rate.

It should be borne in mind that the principal function of the endosperm is to provide nutrients for the initial stages of embryo growth. The nutrients absorbed from the endosperm by the embryo are used largely for the production of new tissue rather than for storage, especially during the early period of embryo growth when the cotyledons are elongating. Nutrients used in the storage of food are derived principally from the tree by absorption via the seed coat. Thus, under normal conditions the endosperm provides the necessary nutritional environment for the first stages of embryo growth, but because of its fragile, turgid qualities it also may be the cause of nut failure.

The most important changes in embryo development that affect the formation of well-filled kernels are the last stages of cotyledon thickening, which occur 14 to 21 days prior to harvest. Finch and Van Horn (*5, p. 442*) concluded:

> The rate at which the filling process proceeds, i. e., the rate at which the carbohydrate containing gel is formed in the nut and converted to fats, bears an important relation to the character of filling attained at harvest time.

They outlined the growth-rate conditions under which well-filled kernels and poorly filled kernels may be produced. The writer believes that Finch and Van Horn overestimated the importance of the endosperm in its relation to cotyledon thickening. It is probable that the carbohydrate-containing endosperm nourishes the embryo in its early stages of growth as it does in most seeds. However, the endosperm in the pecan ovule is largely absorbed before cotyledon thickening is complete, as is shown in figure 3, *C*, *a* and *c*. It seems highly improbable that the enormous quantities of stored food translocated to the cotyledons during the later stages of filling are derived from the endosperm through the process of conversion of gel to solid material, as suggested by Finch and Van Horn. The endosperm is so greatly reduced during the last month of filling as to be of little nutritional significance. This final period of cotyledon thickening is the most important phase of nut filling, as the production of solid kernels at maturity depends upon the complete filling of the cavity within the folds of the cotyledon halves.

The important point here is that sufficient elaborated food must be available from the tree directly, or indirectly from temporary reserves in other parts of the fruit, to fill all of the storage cells developed by the subepidermal meristems. The meristems of the cotyledons function in direct response to the nutrient supply, because the inner meristem is more active at first while the endosperm is still of nutritional significance and the outer meristem builds most of the tissue of the cotyledons during the later stages of filling when nutrients are absorbed from the seed coat. In view of this relation between nutrients and meristem activity, it seems likely that short kernels may be due to poor nutritional conditions in the tree at or prior to the time of cotyledon elongation. Thus, both elongation and thickening of the kernel may be affected by the food supply and the water needed to provide favorable conditions for translocation from the tree.

Finch and Van Horn (*5, p. 445*) stated:

> The problem of reducing or eliminating nuts having the hollow kernel at harvest time is then one of providing for a rapid and continuous progression through the stages of filling. The relation of carbohydrate supply to filling seems of

increased importance now with direct evidence that the solid content of the kernel is formed by conversion from a carbohydrate material that is successively formed within the kernel.

Finch and Van Horn were correct in concluding that filling should be rapid and continuous, but they were in error as to the method of kernel formation. The cotyledons are not formed by conversion from a carbohydrate material (endosperm), but they develop in both length and thickness by means of internal cell division and cell enlargement. During the later stages of filling, stored food enters the tissue of the cotyledon by absorption from the seed coat, since the endosperm at this time is so greatly reduced as to be of little nutritional significance. Finch and Van Horn were correct in stating that the problem of eliminating nuts having the hollow kernel at harvest is one of providing a continuous and adequate carbohydrate supply, but in the later stages of filling this supply moves directly to the cotyledons from the seed coat instead of first appearing as endosperm and later being converted to cotyledonary tissue.

SUMMARY

Pistillate flowers of the pecan are pollinated about June 1 in Maryland, and union of the egg and male gamete occurs about 4 days later. Union of the polar-fusion nucleus and the second male gamete from the pollen tube to form the primary endosperm nucleus likewise takes place a few days (4) after pollination.

The development of the fruit may be divided into two periods characterized as follows: (1) Period of endosperm development, which continues during the second half of June and July and August; and (2) period of embryo growth, which occurs during September, October, and the first week of November. The beginning of shell hardening at the tip of the nut is a morphological feature that separates approximately the two periods.

Endosperm growth begins 14 days after pollination and the endosperm is free-nucleate until about August 1. A large central vacuole filled with liquid is characteristic of the endosperm throughout its development. Beginning early in August, a layer of cellular endosperm, jellylike in nature, is laid down on the outer margins of the central vacuole and adjacent to the seed coat. In September the endosperm is rapidly surrounded and absorbed by the folds of the cotyledons.

Embryo growth begins 42 days after pollination with the first division of the zygote about July 12. By the last of August, the embryo is about 2 mm. long and large enough to be seen with the unaided eye. Growth in size of the ovule and fruit is about complete at this time, and the tip of the shell at the blossom end of the fruit has begun to harden.

The cotyledons elongate into the lower portion of the ovule during the first half of September.

The most important phase of pecan-nut filling is thickening of the cotyledons, which occurred in Maryland between the middle of September and harvest. New tissue is formed by a subepidermal meristem, at first on the inner surface of the cotyledons while the endosperm is supplying nutrients and later on the outer surface during which time nutrients are supplied through the adjacent seed coat. In kernels that

are well-filled and solid, the subepidermal meristem remains active and builds new cells for storage tissue until almost harvesttime. Since meristem activity in the cotyledons seems to function in response to nutrient supply, the formation of plump, solid kernels depends upon the translocation of adequate quantities of food materials from the tree through the seed coat to the embryo during the last month of filling.

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APPARENT DIGESTIBILITY BY SHEEP OF LIGNIN IN PEA AND LIMA-BEAN VINES[1]

By R. E. DAVIS, *nutritionist*, C. O. MILLER, *junior chemist*, and I. L. LINDAHL, *junior chemist, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture*[2]

INTRODUCTION

This paper reports the results of the second part of a study of the composition and apparent digestibility by sheep of pea and lima-bean vines[3] that were preserved by artificial dehydration. The first part of the investigation was concerned primarily with hemicellulose and related compounds (*11*);[4] this paper has to do with the apparent digestibility of lignin.

WORK OF OTHER INVESTIGATORS

It is generally accepted that lignin is the least digestible portion of the structural constituents of plant material (*7*). Some investigators claim that lignin is in part digested by animals, others maintain that it is undigested. Csonka and coworkers (*4*) concluded, from an experiment in which alkali lignin was fed to cows and dogs, that lignin was at least partly broken down by the digestive processes of the animal body. Maynard (*9*) found that digestion of lignin by rabbits and guinea pigs fed alfalfa hay was practically nil, but that a lamb digested 28 percent of the lignin from the same hay. Louw (*7*) reported that with sheep the digestibility coefficient of lignin was 24.5 in grass of 1 month's growth, whereas in grass of 4 months' growth it was only 11.6. In a later experiment in which hay was fed to sheep at daily levels of 600, 800, and 1,000 gm., Louw (*8*) found that the digestibility coefficients were 15.6, 12.4, and 16.4, respectively. Lancaster (*6*) obtained digestibility coefficients ranging from −40.5 for a sample of rape to +32.4 for turnips, in a series of metabolism trials with sheep. In a metabolism experiment in which several different forage plants were fed to sheep, Bondi and Meyer (*1*) obtained di-

[1] Received for publication February 6, 1946.

[2] The authors express their thanks to N. R. Ellis of the Bureau of Animal Industry for assistance during the course of these investigations.

[3] As used in this paper the terms "pea vines" and "lima-bean vines" include the vines and empty pods obtained as byproducts from canneries.

[4] Italic numbers in parentheses refer to Literature Cited, p. 287.

gestibility coefficients that ranged from 35.1 to 64, indicating that the sheep digested lignin comparatively well. Among the investigators who reported that lignin is indigestible are Rogozinski and Starzewska (*12*) and Naumann (*10*). Crampton and Maynard (*3*) recovered 97.8 and 99.3 percent of the dietary lignin in the feces of rabbits and a steer, respectively, and in a later paper Crampton (*2*) reported that lignin was not only poorly digested but also that it interfered with the digestibility of other constituents of the plant material.

MATERIAL AND METHODS

The pea and lima-bean vines used in this study were obtained from a cannery in Pennsylvania. They were dehydrated in a commercial dryer and shipped to the Agricultural Research Center at Beltsville, Md.

Lignin determinations were made by the method of Davis and Miller (*5*). This method includes both enzymatic and chemical treatment. The material was first extracted with ether and then digested by pepsin, clarase, and trypsin. The residue from these digestions was analyzed for lignin by the 72-percent sulfuric acid method. The analytical procedure was the same for both feed and feces. Complete analyses for pea and lima-bean vines were reported in the previous paper (*11*).

The feeding trials were conducted during the winter of 1942. Four yearling and 2-year-old Hampshire ewes were used. During a preliminary period alfalfa hay was mixed with the pea and lima-bean vines, but the amount of alfalfa hay was gradually reduced until the entire feed consisted of pea or lima-bean vines. The animals were placed in metabolism cages a few days prior to the experimental period and confined there throughout the test. The experimental period was 10 days. Details of the procedure are given in the previous paper.

EXPERIMENTAL RESULTS

The lignin content of the lima-bean vines was slightly greater than that of the pea vines, the average lignin content being 6.42 and 6.05 percent, respectively. Owing to the fact that the sheep picked over the feed and left the coarser parts, the lignin content of the refused feed was higher in both cases than that of the original feed. This effect was more pronounced in the refused pea vines, which had an average lignin content of 8.2 percent as compared with 7.6 percent for the refused lima-bean vines.

The digestibility data are given in table 1. As shown in the table, the lignin in pea vines was digested more readily by sheep than the lignin in lima-bean vines. In every trial when the same sheep was fed both feeds, the digestibility of the pea-vine lignin was greater. Incidentally, as shown in the table, the average digestibility of the dry matter in the lima-bean vines was slightly higher than in the pea vines.

TABLE 1.—*Digestibility by sheep of dry matter and lignin in pea and lima-bean vines* [1]

Pea vines			Lima-bean vines		
Item	Dry matter	Lignin	Item	Dry matter	Lignin
Sheep No. 6V:			Sheep No. 6V:		
Fed.....grams..	16,000	924.8	Fed.....grams..	16,000	1,012.8
Consumed.....do....	13,046	693.8	Consumed.....do....	11,409	651.9
Digested.....do....	8,274	105.9	Digested.....do....	7,386	27.9
Digested.....percent..	63.4	15.26	Digested.....percent..	64.7	4.28
Sheep No. 17U:			Sheep No. 17U:		
Fed.....grams..	16,000	924.8	Fed.....grams..	16,000	1,012.8
Consumed.....do....	11,218	554.2	Consumed.....do....	9,886	567.1
Digested.....do....	7,354	89.4	Digested.....do....	6,341	64.4
Digested.....percent..	65.5	16.13	Digested.....percent..	64.1	11.36
Sheep No. 18V:			Sheep No. 18V:		
Fed.....grams..	16,000	1,011.2	Fed.....grams..	16,000	1,040.0
Consumed.....do....	13,795	820.5	Consumed.....do....	12,590	784.6
Digested.....do....	8,477	132.4	Digested.....do....	7,908	121.2
Digested.....percent..	61.4	16.14	Digested.....percent..	62.8	15.45
Sheep No. 42V:			Sheep No. 19U:		
Fed.....grams..	16,000	1,011.2	Fed.....grams..	16,000	1,040.0
Consumed.....do....	12,045	671.9	Consumed.....do....	8,817	484.0
Digested.....do....	7,568	117.2	Digested.....do....	5,976	55.6
Digested.....percent..	62.8	17.44	Digested.....percent..	67.8	11.49
Average percent digested by the 4 sheep.	63.3	16.2	Average percent digested by the 4 sheep.	64.8	10.6

[1] Included pods.

As shown also in the table, the sheep consumed a smaller quantity of lima-bean vines than of pea vines, presumably because the stems of the former were coarser and more woody. Less lignin was consumed in the lima-bean vines than in the pea vines in spite of the fact that the percentage of lignin in the former was slightly higher.

SUMMARY

In a study conducted at the Agricultural Research Center, Beltsville, Md., pea vines and lima-bean vines (including the empty pods) obtained as byproducts from a cannery were fed in the dehydrated state to four yearling and 2-year-old Hampshire ewes. The apparent digestibility of lignin in each of these products was determined in a 10-day test period during which these feeds were given to the same group of animals. The digestion coefficients of the lignin in the pea vines was 16.2 percent and that in the lima-bean vines, 10.6 percent.

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U. S. GOVERNMENT PRINTING OFFICE: 1947